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FORM PTO-1390
(REV. 9-2001)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

DX01052K1

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

10/009445

INTERNATIONAL APPLICATION NO.
PCT/US00/12998

INTERNATIONAL FILING DATE
11 May 2000

PRIORITY DATE CLAIMED
13 May 1999

TITLE OF INVENTION

OX2 RECEPTOR HOMOLOGS

APPLICANT(S) FOR DO/EO/US BARCLAY, A Neil, BROWN, Marion H, GORMAN, Daniel M, LANIER, Lewis L, WRIGHT, Gavin J, CHERWINSKI, Holly, PHILLIPS, Joseph H, HOEK, Robert M and SEDGWICK, Jonathan D

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) The submission must include items (5), (6), (9) and (21) indicated below
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31)
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau)
 - b. ☐ has been communicated by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is attached hereto
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5))

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment
15. ☐ A substitute specification
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter 2 and 35 U.S.C. 1.821 - 1.825
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4)
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4)
20. ☒ Other items or information.
Copy of IPER -International Preliminary Examination Report

U.S. APPLICATION NO. (37 CFR 1.5)					
INTERNATIONAL APPLICATION NO.					
PCT/US00/12998					
ATTORNEY'S DOCKET NUMBER DX01052K1					
21. [] The following fees are submitted:					
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):					
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. . . . \$1040.00					
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO . . . \$890.00					
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO . . . \$740.00					
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) . . . \$710.00					
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) . . . \$100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =					
\$ 890.00					
Surcharge of \$130.00 for furnishing the oath or declaration later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).					
\$					
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	8 - 20 =	0	x \$18.00	\$	
Independent claims	4 - 3 =	1	x \$84.00	\$	84 00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$280.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$	974 00
[] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2				\$	
SUBTOTAL =				\$	
Processing fee of \$130.00 for furnishing the English translation later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).					
\$					
TOTAL NATIONAL FEE =				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) \$40.00 per property +					
\$					
TOTAL FEES ENCLOSED =				\$	974.00
				Amount to be refunded:	\$
				charged:	\$
a. [] A check in the amount of \$ _____ to cover the above fees is enclosed					
b. [x] Please charge my Deposit Account No. 19-0365 in the amount of \$ 974.00 to cover the above fees A duplicate copy of this sheet is enclosed.					
c. [x] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0365 A duplicate copy of this sheet is enclosed					
d. [] Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: Immac J Thampoe Patent Dept K-6-1 1990 Schering-Plough Corporation November 13, 2001 2000 Galloping Hill Road Express Mail label Kenilworth, New Jersey 07033-0530 No. EL 664530736 US					
SIGNATURE					
NAME					
REGISTRATION NUMBER					
36322					

JC07 Rec'd PCT/PTO 13 NOV 2001

10/009445

PATENT CASE: DX01052K1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

-----X	:	
In re Application of: Barclay <i>et al.</i>	:	Examiner: To be assigned
	:	
For: OX2 Receptor Homologs	:	Group Art Unit: To be assigned
	:	
Serial No.:	:	
	:	
Filing Date:	:	
-----X	:	

November 13, 2001

Schering-Plough Corporation
Kenilworth, New Jersey 07033

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

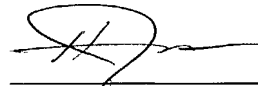
Prior to initial examination on the merits, please amend the subject application as follows:

IN THE SPECIFICATION

On page 1, underneath the title, please enter the following statement:

--The present application is a 35 U.S.C. § 371 National Phase application of PCT/US00/12998, filed May 11, 2000, which claims priority from British Application Number GB 9925989.7, filed November 3, 1999, and British Application Number GB 9911123.9, filed May 13, 1999.--

Respectfully submitted,



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SCHERING-PLOUGH CORPORATION
Patent Department, K-6-1, 1990
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EXPRESS MAIL NO. EL 664530736 US

REMARKS

Enclosed is a write protected floppy diskette with the sequence listing generated by the Patent Office's PATENTIN program. The Diskette should comply with the requirements of 37 CFR §1.824 and is IBM PC compatible with a PC-DOS/MS-DOS operating system. If the diskette has been damaged, please call Applicants and a replacement diskette will be provided. A hard paper copy printout of the diskette is attached thereto.

I hereby state the informational contents of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 CFR 1.821(c) and (e), respectively, are believed to be the same. This submission introduces no new matter, since enclosed sequences are the same as sequences which were submitted in priority documents.

Applicants have invested a significant amount of labor and care in preparing the present submission. The enclosed items are a bona fide effort to bring the present application into full compliance with the rules for sequence submissions. Should this not be the case, Applicants respectfully request notification of specific deficiencies and an opportunity for remedy, as described in 37 CFR 1.135(c).

Applicants believe that no fees are required; however, if any fees are required by the present Response, the Commissioner is authorized to charge any fees or credit any overpayment to DNAX Research Institute Deposit Account No. 04-1239.

Respectfully submitted,

Date: August 18, 2002

By: Sheela Mohan-Peterson
Sheela Mohan-Peterson
Attorney for Applicants
Reg. No. 41,201

enclosures and attachments:
one write-protected diskette (CRM) with paper print-out of contents
Copy of previous Sequence Submission

DNAX Research Institute
901 California Avenue
Palo Alto, California 94304-1104
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Fax: (650) 496-1200

MAMMALIAN PROTEINS; RELATED REAGENTS AND METHODS

FIELD OF THE INVENTION

The present invention relates to compositions and methods
5 for affecting mammalian physiology, including immune system
function. In particular, it provides reagents or methods which
may regulate development and/or the immune system. Diagnostic
and therapeutic uses of these materials are also described.

10 BACKGROUND OF THE INVENTION

The OX2 antigen (OX2) is a cell surface protein identified
on a variety of cells including thymocytes, B lymphocytes,
activated T lymphocytes, neurons, endothelial cells, and
follicular dendritic cells. Barclay (1981) Immunology 44:727-
15 736. Sequence analysis indicates that it is a transmembrane
protein containing two extracellular immunoglobulin-like (Ig-
like) domains and a short cytoplasmic domain. Clark, et al.
(1985) EMBO J. 4:113-118. This domain organization is common and
found in many different leukocyte surface proteins. Barclay, et
20 al. (1997) Leucocyte Antigens Factsbook (2d. ed.) Academic Press,
London. These types of proteins often interact with other
proteins on the surfaces of other cells, also having Ig-like
domains.

The distribution of the OX2 antigen is consistent with a
25 hypothesis that OX2 relays a signal through a binding partner,
e.g., the OX2 receptor (OX2R), to cells within the leukocyte
lineage including macrophages, which express the receptor
(Preston, et al. (1997) Eur. J. Immunol. 27:1911-1918) and
possibly other cells of the monocyte-macrophage lineage. Also,
30 the OX2 has been implicated in regulation of various functions of
macrophages. In this scenario, for instance, expression of OX2
on neurons could establish a direct means of communication to the
resident macrophages of the brain called microglia that might
express OX2R, since they originate from the monocyte-macrophage
35 lineage. Perry and Gordon (1988) Trends Neurosci. 11:273-277.

Generally, defective or exaggerated activation of
macrophages contributes to pathogenesis of a wide range of

immunological and other diseases. See, e.g., McGee, et al. (eds. 1992) Oxford Textbook of Pathology Oxford University Press, Oxford; Lewis and McGee (eds. 1992) The Macrophage IRL Press, Oxford; and Bock and Goode (eds. 1997) The Molecular Basis of Cellular Defence Mechanisms Wiley & Sons.

Also, identification of the OX2 interacting proteins, e.g., the OX2R for the OX2 antigen, is difficult because the affinities of the interactions are often very low. This means that the binding of recombinant forms of cell surface proteins, e.g., OX2, to their binding partners, the interacting proteins, e.g., OX2R, is insufficiently stable to allow detection by normal methods. Thus, the interaction between CD48 and CD2, of which both partners contain two Ig-like domains in their extracellular regions, has a half-life of a fraction of a second. See Van der Merwe, et al. (1993) Biochem. Soc. Trans. 21:340S; and Van der Merwe and Barclay (1994) Trends Biochem. Sci. 19:354-358.

Recombinant forms of cell surface proteins such as OX2 can be made multivalent by a number of methods and used to detect novel proteins. An OX2 has been engineered to include a tag of two Ig-like domains from CD4. The recombinant soluble proteins are expressed by conventional expression methods in eukaryotic cells. In an earlier study, an interaction was observed between the multivalent recombinant OX2 protein on fluorescent beads and mouse macrophages. Preston, et al. (1997) Eur. J. Immunol. 27:1911-1918.

Despite the above, attempts to identify OX2R on mouse macrophages through use of a blocking antibody OX89 were not successful. Preston, et al. (1997) Eur. J. Immunol. 27:1911-1918.

From the foregoing, it is evident that the discovery, identification, and understanding of novel receptors for OX2-like molecules would be highly advantageous. The present invention provides new receptor homologs for OX2 ligands and related compounds, and methods for their use.

SUMMARY OF THE INVENTION

The present invention is directed to novel receptor homologs, for the ligand designated OX2, e.g., rodent and primate
embodiments. These have been designated generically OX2 receptor
5 homologs (OX2RH), with embodiments from various rodent and
primate species. Two have been established as actually binding
to the respective species OX2. In particular, it provides
description of homologs designated OX2RH1, OX2RH2, OX2RH3, and
OX2RH4. It includes nucleic acids encoding the polypeptides
10 themselves and methods for their production and use. The nucleic
acids of the invention are characterized, in part, by their
homology to cloned complementary DNA (cDNA) sequences enclosed
herein.

The present inventors have produced a new monoclonal
15 antibody (mAb), designated OX102, for OX2R on rat macrophages
which blocks the interaction between OX2 and OX2R. They have
also isolated and characterised the rat OX2R gene and
polypeptide. Sequences for the rat OX2R nucleic acid molecule
and polypeptide (predicted amino acid sequence) are provided
20 herein. By analogy with similar proteins, the inventors teach
that the nucleotide and amino acid sequences of human OX2R will
be at least 50% homologous with the corresponding rat OX2R
sequences. The availability of the rat OX2R cDNA and a predicted
OX2R polypeptide sequence enable identification of the equivalent
25 human OX2R sequences, either through screening of known human
sequences or the isolation of human nucleic acids by
hybridisation or PCR technology.

The presence of a large cytoplasmic sequence in the OX2R
polypeptide indicates that OX2R has a role in macrophage
30 function, either in signalling or through interactions with
components of the cytoplasm. Thus, the present invention
provides for reagents based on OX2R that either mimic or
recognise OX2R polypeptide or nucleic acid sequences, e.g., small
molecular entities designed to react with OX2R binding sites,
35 mAbs raised against OX2R or antisense sequences, which reagents
constitute therapeutically useful compounds for modifying the

in Table 1), which fragment exhibits a characteristic property of full-length OX2R protein. For example, the fragment may bind with an OX2 protein or with an antibody for full-length OX2R protein. In one embodiment, the fragment includes part or all of the cytoplasmic domain of OX2R or an active portion of that domain. In another embodiment, the fragment includes part or all of the extracellular domain of OX2R or an active portion of that domain. Since the extracellular domain is believed to be responsible for interacting with OX2, such fragments according to the present invention including part or all of the extracellular domain, can be used to screen candidate compounds for an ability to interfere with the binding between OX2 and OX2R.

Accordingly, the present invention provides methods and materials for screening candidate compounds likely to have the ability to interfere with the OX2/OX2R interaction between macrophages and other cells, including thymocytes, B lymphocytes, activated T lymphocytes, neurons, endothelial cells and follicular dendritic cells.

Polypeptides and fragments as above may be recombinant and/or isolated polypeptides.

In a further aspect, the present invention provides a substance comprising a nucleic acid having the nucleotide sequence of Table 1. The present invention also provides a substance which comprises a nucleic acid molecule encoding an above polypeptide or fragment. Thus, Table 1 shows the cDNA sequence of an exemplary nucleic acid molecule coding for an OX2R polypeptide. The nucleic acid molecule may have at least 50% sequence homology with the nucleic acid sequence of Table 1.

The invention also provides a substance comprising a nucleic acid molecule having part of a coding nucleotide sequence of Table 1. Where the substance comprises a part of a coding nucleotide sequence of Table 1, it will be a part which is characteristic of an OX2R gene. Thus, the part may encode a polypeptide fragment as stated above, which binds with OX2 or an antibody for full-length OX2R. Alternatively, the part may comprise at least 4 to 7 contiguous codons, often at least 7 to 9

contiguous codons, typically at least 9 to 13 contiguous codons and, most preferably, at least 20 to 30 contiguous codons of a nucleotide sequence of Table 1. Alternatively, the part may encode at least 4 to 7 contiguous amino acids, often at least 7 to 9 contiguous amino acids, typically at least about 9 to 13 contiguous amino acids and, most preferably, at least about 20 to 30 contiguous amino acids of a polypeptide sequence of Table 1.

Nucleic acid molecules as above may be recombinant and/or isolated.

In further aspects, the present invention provides vectors comprising an OX2R nucleic acid as herein provided, e.g., expression vectors in which an OX2R nucleic acid sequence is operably linked to control sequences to direct its expression. Also provided are host cells transformed with such vectors. The present invention further includes a method of producing OX2R polypeptides, comprising culturing such host cells and isolating OX2R polypeptide produced.

In a further aspect, the present invention provides a method of expressing OX2R in host cells, the method including the steps of inserting a nucleic acid molecule as above into a host cell and providing conditions for expression of said nucleic acid molecule in the host cell. The method may employ an expression vector.

In a further aspect, the present invention provides a composition comprising a soluble form of an OX2R polypeptide or fragment as above, the composition also optionally including an adjuvant, pharmaceutical carrier, or excipient. The composition can be used, e.g., to generate an antibody response to an OX2R polypeptide.

In further aspects, the present invention provides above OX2R polypeptides and nucleic acid molecules for use in screening candidate compounds likely to be useful as therapeutics. The present invention provides the use of an OX2R polypeptide or fragment as above in the screening for substances likely to be useful for the treatment of bacterial infections, autoimmune diseases, and the like.

The present invention also provides the use of OX2R polypeptides, polypeptide fragments, and nucleic acids for the identification of ligands for OX2R other than OX2. The present invention also provides the use of OX2R polypeptides, polypeptide
5 fragments, and nucleic acids for the design of mimetics of OX2.

In a further aspect, the present invention provides antibodies capable of specifically binding to OX2R polypeptides, polypeptide fragments, and nucleic acids as above, and compositions comprising such antibodies. These antibodies can be
10 used in assays to detect and quantify the presence of OX2R, as well as in methods of purifying OX2R. The antibodies may be polyclonal. Preferably, the antibodies are IgG antibodies, more preferably monoclonal IgG antibodies.

In a further aspect, the present invention provides the use
15 of OX2R polypeptides, polypeptide fragments, and nucleic acid molecules as provided herein to produce binding molecules, such as substances with one or more antibody domains, which can block the interaction between OX2 and OX2R. These may be included in a composition likely to be useful in the preparation of medicaments
20 for the treatment of bacterial infections, autoimmune diseases, and the like. Where the binding molecules are antibodies, they may be IgG antibodies, preferably monoclonal IgG antibodies.

In a further aspect, the present invention provides the use
25 of OX2R nucleic acids as defined above in the design of antisense oligonucleotides to restrict OX2R expression in a population of macrophage cells, e.g., phosphorothiolated or cholesterol-linked oligonucleotides which can facilitate internalization and stabilization of the oligonucleotides.

In a further aspect, the present invention provides a method
30 of amplifying a nucleic acid test sample, which comprises priming a nucleic acid polymerase reaction with primer oligonucleotides obtainable from the sequence information provided herein. The nucleic acid test sample may be of a human, such that nucleic acid coding for a human OX2R is amplified using such a method.

35 In a further aspect, the present invention provides a method of obtaining a nucleic acid molecule coding for part or all of an

OX2R from a species other than rat, e.g., human OX2R, which comprises probing a nucleic acid test sample from the species of interest with a nucleic acid probe obtainable from the sequence information provided herein.

5 In a further aspect, the present invention provides a method of obtaining an OX2R polypeptide sequence from a species other than rat, e.g., a human OX2R, which comprises searching databases for polypeptide sequences at least 50% homologous with an OX2R amino acid sequence as provided herein (Table 1). Similarly,
10 OX2R nucleic acid sequences from species other than rat, e.g., human OX2R, can be obtained by searching databases for nucleotide sequences at least 50% homologous with an OX2R nucleotide sequence as provided herein.

15 The present invention also provides the use of the nucleic acid sequence information provided herein in the search for mutations in OX2R genes, e.g., using techniques such as single stranded conformation polymorphism (SSCP).

The present invention provides a composition of matter selected from: a substantially pure or recombinant polypeptide
20 comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 2; a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 2; a natural sequence rodent
25 OX2RH1 polypeptide comprising mature SEQ ID NO: 2; a fusion polypeptide comprising rat OX2RH1 sequence; a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 4; a substantially pure or recombinant
30 polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 4; a natural sequence rodent OX2RH1 polypeptide comprising mature SEQ ID NO: 4; a fusion polypeptide comprising human OX2RH1 sequence; a substantially pure or recombinant
35 polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of

SEQ ID NO: 6; a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 6; a natural sequence rodent OX2RH1 polypeptide comprising mature SEQ ID NO: 6; a fusion polypeptide comprising mouse OX2RH1 sequence; a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 8; a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 8; a natural sequence rodent OX2RH1 polypeptide comprising mature SEQ ID NO: 8; a fusion polypeptide comprising human OX2RH2 sequence; a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 10; a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 10; a natural sequence rodent OX2RH2 polypeptide comprising mature SEQ ID NO: 10; a fusion polypeptide comprising mouse OX2RH2 sequence; a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 12; a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 12; a natural sequence rodent OX2RH3 comprising mature SEQ ID NO: 12; a fusion polypeptide comprising mouse OX2RH3 sequence; a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 20; a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 20; a natural sequence primate OX2RH1.2 polypeptide comprising mature SEQ ID NO: 20; a fusion polypeptide comprising primate OX2RH1.2 sequence; a

substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 23; a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 23; a natural sequence rodent OX2RH4 polypeptide comprising mature SEQ ID NO: 23; or a fusion polypeptide comprising mouse OX2RH4 sequence. Some preferred embodiments include wherein the distinct nonoverlapping segments of identity: include one of at least eight amino acids; include one of at least four amino acids and a second of at least five amino acids; include at least three segments of at least four, five, and six amino acids, or include one of at least twelve amino acids. Other preferred embodiment include those wherein the: a) OX2RH1 polypeptide: comprises a mature sequence of Tables 1 or 2; is an unglycosylated form of OX2RH polypeptide; is from a primate, such as a human; is from a rodent, such as a rat or mouse; comprises at least seventeen amino acids of SEQ ID NO: 2, 4, 6, or 20; exhibits at least four nonoverlapping segments of at least seven amino acids of SEQ ID NO: 2, 4, 6, or 20; is a natural allelic variant of OX2RH1; has a length at least about 30 amino acids; exhibits at least two non-overlapping epitopes which are specific for a primate or rodent OX2RH1; is glycosylated; has a molecular weight of at least 30 kD with natural glycosylation; is a synthetic polypeptide; is attached to a solid substrate; is conjugated to another chemical moiety; is 5-fold or less substituted from natural sequence; or is a deletion or insertion variant from a natural sequence; b) OX2RH2 polypeptide: comprises a mature sequence of Table 2; is an unglycosylated form of OX2RH2 polypeptide; is from a primate, such as a human; is from a rodent, such as a mouse; comprises at least seventeen amino acids of SEQ ID NO: 8 or 10; exhibits at least four nonoverlapping segments of at least seven amino acids of SEQ ID NO: 8 or 10; is a natural allelic variant of OX2RH2; has a length at least about 30 amino acids; exhibits at least two non-overlapping epitopes which are specific for a primate or rodent OX2RH2; is

glycosylated; has a molecular weight of at least 30 kD with natural glycosylation; is a synthetic polypeptide; is attached to a solid substrate; is conjugated to another chemical moiety; is a 5-fold or less substitution from natural sequence; or is a deletion or insertion variant from a natural sequence; c) OX2RH3 polypeptide: comprises a mature sequence of Table 3; is an unglycosylated form of OX2RH3; is from a rodent, such as a mouse; comprises at least seventeen amino acids of SEQ ID NO: 12; exhibits at least four nonoverlapping segments of at least seven amino acids of SEQ ID NO: 12; is a natural allelic variant of OX2RH3; has a length at least about 30 amino acids; exhibits at least two non-overlapping epitopes which are specific for a rodent OX2RH3; is glycosylated; has a molecular weight of at least 30 kD with natural glycosylation; is a synthetic polypeptide; is attached to a solid substrate; is conjugated to another chemical moiety; is 5-fold or less substituted from natural sequence; or is a deletion or insertion variant from a natural sequence; or d) OX2RH4 polypeptide: comprises a mature sequence of Table 2; is an unglycosylated form of OX2RH4; is from a rodent, such as a mouse; comprises at least seventeen amino acids of SEQ ID NO: 23; exhibits at least four nonoverlapping segments of at least seven amino acids of SEQ ID NO: 23; is a natural allelic variant of OX2RH4; has a length at least about 30 amino acids; exhibits at least two non-overlapping epitopes which are specific for a rodent OX2RH4; is glycosylated; has a molecular weight of at least 30 kD with natural glycosylation; is a synthetic polypeptide; is attached to a solid substrate; is conjugated to another chemical moiety; is 5-fold or less substituted from natural sequence; or is a deletion or insertion variant from a natural sequence. In yet other embodiments, the invention provides a composition comprising: a1) a substantially pure OX2RH1 and another Ig superfamily member; a2) a substantially pure OX2RH2 and: another Ig superfamily member, DAP12, or DAP10; a3) a substantially pure OX2RH3 and: another Ig superfamily member, DAP12, or DAP10; a4) a substantially pure OX2RH4 and: another Ig superfamily member, DAP12, or DAP10; or a

sterile OX2RH1 polypeptide; a sterile OX2RH2 polypeptide; a sterile OX2RH3 polypeptide; a sterile OX2RH4 polypeptide; the OX2RH1, OX2RH2, OX2RH3, or OX2RH4 polypeptide and a carrier, wherein the carrier is an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration.

Fusion polypeptides are also provided, e.g., comprising: mature protein sequence of Tables 1-3; a detection or purification tag, including a FLAG, His6, or Ig sequence; or sequence of another Ig superfamily protein. Kits are also provided, e.g., comprising an OX2RH polypeptide and: a compartment comprising the protein or polypeptide; or instructions for use or disposal of reagents in the kit.

The invention also embraces various antibody like reagents, including antibodies derived from different species. It provides, e.g., a binding compound comprising an antigen binding site from an antibody, which specifically binds to a natural OX2RH polypeptide, e.g., OX2RH1, OX2RH2, OX2RH3, and/or OX2RH4, wherein: the binding compound is in a container; the OX2RH polypeptide is from a rodent or primate; the binding compound is an Fv, Fab, or Fab2 fragment; the binding compound is conjugated to another chemical moiety; or the antibody: is raised against a peptide sequence of a mature polypeptide of Tables 1-3; is raised against a mature OX2RH; is raised to a purified mammalian OX2RH; is immunoselected; is a polyclonal antibody; binds to a denatured OX2RH; exhibits a K_d to antigen of at least 30 μM ; is attached to a solid substrate, including a bead or plastic membrane; is in a sterile composition; or is detectably labeled, including a radioactive or fluorescent label. Kits are thereby provided, e.g., comprising such binding compounds and: a compartment comprising the binding compound; or instructions for use or disposal of reagents in the kit. Methods are also provided, e.g., producing an antigen:binding compound or antigen:antibody complex, comprising contacting under appropriate conditions a mammalian OX2RH polypeptide with an antibody, thereby allowing the complex to form. Preferably, in this method: the complex is

purified from other cytokine or Ig superfamily receptors; the complex is purified from other antibody; the contacting is with a sample comprising a mammalian OX2; the contacting allows quantitative detection of the antigen; the contacting is with a sample comprising the antibody; or the contacting allows quantitative detection of the antibody. Related compositions are made available, e.g., comprising: a sterile binding compound, or the binding compound and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration.

The present invention further provides nucleic acids, e.g., an isolated or recombinant nucleic acid encoding a OX2RH polypeptide wherein the: OX2RH is from a mammal; or the nucleic acid: encodes an antigenic peptide sequence of Tables 1-3; encodes a plurality of antigenic peptide sequences of Tables 1-3; exhibits identity over at least thirteen nucleotides to a natural cDNA encoding the segment; is an expression vector; further comprises an origin of replication; is from a natural source; comprises a detectable label; comprises synthetic nucleotide sequence; is less than 6 kb, preferably less than 3 kb; is from a primate or rodent; comprises a natural full length coding sequence; is a hybridization probe for a gene encoding the OX2RH; further encodes DAP12 or DAP10; or is a PCR primer, PCR product, or mutagenesis primer. Cells comprising the recombinant nucleic acid are also provided, e.g., wherein the cell is: a prokaryotic cell; a eukaryotic cell; a bacterial cell; a yeast cell; an insect cell; a mammalian cell; a mouse cell; a primate cell; or a human cell. Kits comprising the nucleic acid are provided, e.g., with a compartment comprising the nucleic acid; with a compartment further comprising a mammalian OX2RH polypeptide; or with instructions for use or disposal of reagents in the kit.

Alternatively, the invention provides a nucleic acid which: hybridizes under wash conditions of 30 minutes at 40° C and less than 2M salt to the coding portion of SEQ ID NO: 1, 3, 5, 7, 9, 11, 19, or 22; or exhibits identity over a stretch of at least

about 30 nucleotides to a primate or rodent OX2RH cDNA.
Preferably, the wash conditions are at: 50° C and/or 500 mM salt;
or 60° C and/or 150 mM salt; the stretch is at least 55
nucleotides or 75 nucleotides; or the nucleic acid further
5 encodes a DAP12 or DAP10 peptide.

Other methods are further embraced, e.g., a method of
modulating physiology or development of a cell or tissue culture
cells comprising contacting the cell with an agonist or
antagonist of a mammalian OX2RH. Often, the cell is transformed
10 with a nucleic acid encoding an OX2RH.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

OUTLINE

- I. General
- 5 II. Activities
- III. Nucleic acids
 - A. encoding fragments, sequence, probes
 - B. mutations, chimeras, fusions
 - C. making nucleic acids
 - 10 D. vectors, cells comprising
- IV. Proteins, Peptides
 - A. fragments, sequence, immunogens, antigens
 - B. muteins
 - C. agonists/antagonists, functional equivalents
 - 15 D. making proteins
- V. Making nucleic acids, proteins
 - A. synthetic
 - B. recombinant
 - C. natural sources
 - 20 VI. Antibodies
 - A. polyclonals
 - B. monoclonal
 - C. fragments; Kd
 - D. anti-idiotypic antibodies
 - 25 E. hybridoma cell lines
- VII. Kits and Methods to quantify OX2RHs
 - A. ELISA
 - B. assay mRNA encoding
 - C. qualitative/quantitative
 - 30 D. kits
- VIII. Therapeutic compositions, methods
 - A. combination compositions
 - B. unit dose
 - C. administration
 - 35 IX. Screening
 - X. Ligands

I. General

40 The present invention provides amino acid sequences and DNA sequences of mammalian, herein primate and rodent, receptor-like subunit molecules, these designated OX2 receptor homologs (OX2RH). These genes have particular defined properties, either or both structural and biological. Various cDNAs encoding these molecules were obtained from mammal, e.g., human and rodent, cDNA

sequence libraries. Other mammalian, e.g., primate, rodent, or other, counterparts would also be desired.

The OX2 antigen was first characterized in rat, using a monoclonal antibody (mAb) MRC OX2. See, e.g., McMaster and Williams (1979) Eur. J. Immunol. 9:426-433; Barclay (1981) Immunology 44:727-736; Barclay (1981) Immunology 42:593-600; Bukovsky, et al. (1984) Immunology 52:631-640; and Webb and Barclay (1984) J. Neurochem. 43:1061-1067. Using this antibody in immunohistochemical (IHC) staining of tissue sections or cell suspensions for flow cytometry revealed that the OX2 antigen was expressed by a wide variety of cells, e.g. neurons, vascular endothelium, B cells, activated T cells, follicular dendritic cells, smooth muscle cells and trophoblasts. Furthermore, human OX2 is known to be expressed in normal brain and by B cells. McCaughan, et al. (1987) Immunogenetics 25:329-335.

Characterization of the rat protein recognized by MRC OX2 (Clark, et al. (1985) EMBO J. 4:113-118) revealed that OX2 consists of about 248 amino acids comprising two extracellular immunoglobulin (Ig) domains, a transmembrane domain and a short C-terminal cytoplasmic tail. The molecule is glycosylated through 6 N-linked glycosylation sites, three of which are present in the N-terminal V-like Ig domain and the others reside in the membrane proximal C2-like Ig domain. This places OX2 in the Ig superfamily (IgSF), forming a sub-group of small IgSF molecules with molecules like CD2, CD48, CD58, CD80, CD86, CD90, and CD147, which are characterized structurally, e.g., by the existence of the immunoglobulin-like domains corresponding to Ig variable and constant domains, a transmembrane segment, an intracellular domain, and characteristic cysteine and tryptophan residue spacings. See, e.g., Campbell, et al. (1979) Nature 282:341-342. Interestingly, CD90 is also highly expressed by neurons. Williams, et al. (1977) Cold Spring Harb. Symp. Quant. Biol. 41 Pt 1:51-61. Furthermore, it was shown that OX2 was a structural homologue of CD80 and CD86 (Borriello, et al. (1997) J. Immunol. 158:4548-4554) and that the OX2 gene was closely linked to those coding for CD80 and CD86 on chromosome 16 in the mouse.

Borriello, et al. (1998) Mamm. Genome 9:114-118. Both CD80 and CD86 serve as ligands in a process known as co-stimulation, and therefore it is likely that OX2 would act as a ligand as well. The OX2 antigen will be referred hereafter as the OX2 protein or
 5 ligand OX2. The binding partner will be referred to as the OX2 receptor.

To identify the receptor for OX2 (OX2R), a multivalent reagent was prepared using rat OX2-rat CD4 fusion protein bound to fluorescent beads. This reagent was shown to bind to mouse
 10 and rat peritoneal macrophages, and this binding could be blocked by the mAb MRC OX88. Preston, et al. (1997) Eur. J. Immunol. 27:1911-1918. This mAb was shown to bind to macrophages isolated from both peritoneum and spleen and in IHC on spleen sections staining was found in areas known to contain high proportions of
 15 macrophages.

A second monoclonal antibody raised by the Barclay group, designated OX102, was shown to bind macrophages in the rat species and also to prevent specifically the binding of the OX2 molecule to rat peritoneal macrophages. Isolation of material
 20 binding to the OX102 molecule and N-terminal sequencing showed the putative OX2 receptor (OX2R) to be a novel molecule. This was cloned as described herein. That the protein recognized by the OX102 antibody was indeed the receptor was supported by the demonstration of a longer cytoplasmic tail on this molecule
 25 relative to the OX2 molecule itself (the ligand). Clark, et al. (1985) EMBO J. 4:113-118. Preliminary analysis of the OX2R did not reveal obvious motifs consistent with known signaling molecules although this does not exclude the potential role of this molecule in mediating OX2-delivered signals.

30 Then, a mouse homolog was identified, designated OX2RH1. Because the terminology OX2R should be reserved for those proteins which have been verified to actually bind to the OX2, the initial designation applied is a receptor homolog of the group 1. The nucleotide and amino acid sequences of this
 35 molecule are described herein.

Further analysis of available sequence databases revealed the presence of another distinct form of OX2RH, a molecule that showed significant homology in the putative extracellular Ig-domain structures with OX2RH1 but with a different transmembrane and cytoplasmic sequence. These forms have herein been designated OX2RH2, and both human and mouse embodiments have been identified. Of particular note is the presence of a lysine (K) moiety at positions 224 (human) and 170 (mouse) that lies within the transmembrane portion of the molecule. Such a residue suggests that this molecule will associate with molecular partners such as DAP12 known to express motifs capable of signaling for cellular activation. See, e.g., Lanier, et al. (1998) Nature 391:703-707; Colonna(1998) Nature 391:642-3; Campbell, et al. (1999) Int. J. Biochem. Cell. Biol. 31:631-636; and Lopez-Botet, et al. (1999) Curr. Opin. Immunol. 11:301-307. Moreover, such suggests various signaling pathways and associated biochemistry. See, e.g., Lanier, et al. (1998) Immunity 8:693-701; Smith, et al. (1998) J. Immunol. 161:7-10; Gosselin, et al. (1999) J. Leukoc. Biol. 66:165-171; Tomasello, et al.(1998) J. Biol. Chem. 273:34115-34119; and McVicar, et al. (1998) J. Biol. Chem. 273:32934-32942. But, a full length mouse or human OX2RH2 form is yet to be isolated.

There is high homology between the mouse and rat extracellular regions of the OX2RH1 molecule, both of which have been confirmed to bind to their respective species OX2. Thus, the rat and mouse OX2RH1 embodiments are properly also referred to functionally as OX2R. Both contain typical extracellular, transmembrane, and intracellular domain structures. Human OX2RH1 embodiments were discovered. Additionally, soluble forms of the rat and mouse OX2RH1 may exist.

Related homologs, designated OX2RH2 and OX2RH4, have also been described, various embodiments originating in mouse and human. OX2RH2, H3, and H4 embodiments exhibit a charged lysine residue in the transmembrane segment. The human OX2RH2 embodiment lacks a signal sequence and shows some genomic sequence earmarks, suggesting that the functional form of the

natural human OX2RH2 should be closely related but slightly different from the sequence provided. The functional relationship of the mouse and human homologs 2 and 4 remain to be confirmed.

5 A further OX2R homolog was also found in the mouse. Although its homology is much more divergent, it exhibits some similarities in sequence. In particular, it has a lysine residue in the transmembrane region. Thus, like the other OX2RH2, H3, and H4 molecules exhibiting this feature, it would be expected to
10 signal via an associating molecule such as DAP12. This embodiment is herein designated OX2RH3 from rodent, e.g., mouse.

Ongoing analysis of the expression patterns of the OX2RH1 indicates that in rat, mouse, and human leukocytes, OX2RH1 (as determined by flow cytometric staining with the OX102 antibody
15 and/or analysis of mRNA expression by PCR techniques) is expressed most strongly by monocytes, granulocytes, and mast cells, marginally by B cells, and weakly by T cells. This is consistent with the preferential binding of the ligand OX2 to macrophages in earlier studies. In the normal rat central
20 nervous system, a proportion of resident macrophage (or microglial cells) also express the OX2R, but at a low level.

Some applicable standard methods are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring
25 Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols. 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and periodic supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York; each of which is
30 incorporated herein by reference.

Nucleotide (SEQ ID NO: 1) and corresponding amino acid sequence (SEQ ID NO: 2) of a rodent, e.g., rat, OX2 receptor homolog 1 (OX2RH1) coding segment is shown in Table 1. Similarly, further embodiments, primate, e.g., human, and rodent,
35 e.g., mouse, are described, designated OX2RH1, 1.2, 2, and 4. The nucleic acid sequences are SEQ ID NO: 3, 19, 5, 7, 9, and 22;

the corresponding amino acid sequences are SEQ ID NO: 4, 20, 6, 8, 10, and 23 which are presented in Table 2. Table 3 provides the sequence of other rodent, e.g., mouse, OX2RH3 (SEQ ID NO: 11 and 12).

- 5 Reverse translation nucleic acid sequences are provided in Table 4 (SEQ ID NO: 13-14, 21, 15-17, 24, and 18). Table 5 provides alignment and numeric comparison of polypeptide sequences.

Table 1: Nucleotide and polypeptide sequences of rodent OX2R (homolog 1).

rat OX2RH1 (SEQ ID NO: 1 and 2):

5	agcggaggga tcctgggtcat ggtcaccgct gctcccctac ctgtgaagag aaagagcacc																60
	gagtgagccg ctgaaaacca gaaaaccgaa atg ctc tgc ttt tgg aga act tct																114
	Met Leu Cys Phe Trp Arg Thr Ser																
10	-20																
	cac gta gca gta ctc ttg atc tgg ggg gtc ttc gcg gct gag tca agt	162															
	His Val Ala Val Leu Leu Ile Trp Gly Val Phe Ala Ala Glu Ser Ser																
	-15			-10			-5			-1							
15	tgt cct gat aag aat caa aca atg cag aac aat tca tca act atg aca	210															
	Cys Pro Asp Lys Asn Gln Thr Met Gln Asn Asn Ser Ser Thr Met Thr																
	1	5				10			15								
20	gaa gtt aac act aca gtg ttt gta cag atg ggt aaa aag gct ctg ctc	258															
	Glu Val Asn Thr Thr Val Phe Val Gln Met Gly Lys Lys Ala Leu Leu																
	20						25			30							
25	tgc tgc cct tct att tca ctg aca aaa gta ata tta ata aca tgg aca	306															
	Cys Cys Pro Ser Ile Ser Leu Thr Lys Val Ile Leu Ile Thr Trp Thr																
	35			40			45										
30	ata acc ctc aga gga cag cct tcc tgc ata ata tcc tac aaa gca gac	354															
	Ile Thr Leu Arg Gly Gln Pro Ser Cys Ile Ile Ser Tyr Lys Ala Asp																
	50			55			60										
35	aca agg gag acc cat gaa agc aac tgc tcg gac aga agc atc acc tgg	402															
	Thr Arg Glu Thr His Glu Ser Asn Cys Ser Asp Arg Ser Ile Thr Trp																
	65	70			75			80									
40	gcc tcc aca cct gac ctc gct cct gac ctt cag atc agt gca gtg gcc	450															
	Ala Ser Thr Pro Asp Leu Ala Pro Asp Leu Gln Ile Ser Ala Val Ala																
	85			90			95										
45	ctc cag cat gaa ggg cgt tac tca tgt gat ata gca gta cct gac ggg	498															
	Leu Gln His Glu Gly Arg Tyr Ser Cys Asp Ile Ala Val Pro Asp Gly																
	100			105			110										
50	aat ttc caa aac atc tat gac ctc caa gtg ctg gtg ccc cct gaa gta	546															
	Asn Phe Gln Asn Ile Tyr Asp Leu Gln Val Leu Val Pro Pro Glu Val																
	115			120			125										
55	acc cac ttt cca ggg gaa aat aga act gca gtt tgt gag gcg att gca	594															
	Thr His Phe Pro Gly Glu Asn Arg Thr Ala Val Cys Glu Ala Ile Ala																
	130			135			140										
60	ggc aaa cct gct gcg cag atc tct tgg acg cca gat ggg gat tgt gtc	642															
	Gly Lys Pro Ala Ala Gln Ile Ser Trp Thr Pro Asp Glu Asp Cys Val																
	145	150			155			160									
65	gct aag aat gaa tca cac agc aat ggc acc gtg act gtc cgg agc aca	690															
	Ala Lys Asn Glu Ser His Ser Asn Gly Thr Val Thr Val Arg Ser Thr																
	165			170			175										

	tgc	cac	tgg	gag	cag	cac	gtg	tct	gtc	gtg	ttc	tgt	gtt	gtc	tct		738					
	Cys	His	Trp	Glu 180	Gln	Ser	His	Val	Ser 185	Val	Val	Phe	Cys	Val 190	Val	Ser						
5	cac	ttg	aca	act	ggt	aac	cag	tct	ctg	tct	ata	gaa	ctg	ggt	aga	ggg	786					
	His	Leu	Thr 195	Thr	Gly	Asn	Gln	Ser 200	Leu	Ser	Ile	Glu	Leu 205	Gly	Arg	Gly						
10	ggt	gac	caa	tta	tta	gga	tca	tac	att	caa	tac	atc	atc	cca	tct	att	834					
	Gly	Asp 210	Gln	Leu	Leu	Gly	Ser 215	Tyr	Ile	Gln	Tyr	Ile	Ile 220	Pro	Ser	Ile						
15	att	att	ttg	atc	atc	ata	gga	tgc	att	tgt	ctt	ttg	aaa	atc	agt	ggc	882					
	Ile	Ile	Leu	Ile	Ile	Ile	Gly 230	Cys	Ile	Cys	Leu 235	Leu	Lys	Ile	Ser	Gly 240						
20	tgc	aga	aaa	tgt	aaa	ttg	cca	aaa	tcg	gga	gct	act	cca	gat	att	gag	930					
	Cys	Arg	Lys	Cys 245	Lys	Leu	Pro	Lys	Ser	Gly 250	Ala	Thr	Pro	Asp	Ile 255	Glu						
25	gag	gat	gaa	atg	cag	ccg	tat	gct	agc	tac	aca	gag	aag	agc	aat	cca	978					
	Glu	Asp	Glu	Met 260	Gln	Pro	Tyr	Ala	Ser 265	Tyr	Thr	Glu	Lys	Ser 270	Asn	Pro						
30	ctc	tat	gat	act	gtg	acc	acg	acg	gag	gca	cac	cca	gcg	tca	caa	ggc	1026					
	Leu	Tyr	Asp 275	Thr	Val	Thr	Thr	Thr 280	Glu	Ala	His	Pro	Ala 285	Ser	Gln	Gly						
35	aaa	gtc	aat	ggc	aca	gac	tgt	ctt	act	ttg	tca	gcc	atg	gga	atc		1071					
	Lys	Val 290	Asn	Gly	Thr	Asp	Cys 295	Leu	Thr	Leu	Ser	Ala 300	Met	Gly	Ile							
40	tagaaccaag	gaaaagaagt caagagacat cataattact gcttttcttt ctttaaactt					1131															
45	ctccaatgga	gggaaattag ctcttctgaa gttcttagaa agcacaaatg ttctaattgga					1191															
50	tttgcccttta	agttcttcta tcattggaag tttggaatct ttgctgctac ctgttaattc					1251															
55	taggaagaac	tgatttaatt attacaaaga aagcacattg ttatggtaaa atatcaaatt					1311															
60	gtgcaataca	atgatgaaaa ctgagtttcc tcaagaaata actgcagaag gaacaatcat					1371															
65	tactaaagca	tttcatgtga gttcttccaa aaaagaaaat ccctgtgtat acgacatgat					1431															
70	tatggatatgt	gtgtgccttt atatgtttgt ttacaaatgt gtatatatgc acacatctga					1491															
75	ttatcaagac	atctctgtca aaaactcact ggcgttccag atttatgaaa gctaataaag					1551															
80	tgagtattgg	agatgttttt ata					1574															
85	MLCFWRTSHVAVLLIWGVFAAESSCPDKNQTMQNNSSTMTTEVNNTTVFVQMGKKALLCCPSISLTKVILIT																					
90	GQPSCIISYKADTRETHESNCSDRSITWASTPDLAPDLQISAVALQHEGRYSCDIAVPDGNFQNIYDLQV																					
95	THFFPENRTAVCEAIAGKPAAQISWTPDGDCVAKNESHSGTGTVRSTCHWEQSHSVVFVCVSHLTGN																					
100	LGRGGDQLLSGIYIIPSIILLIIIGCICLLKISGCRKCKLPKSGATPDIEEDEMOPYASYTEKSNPLY																					
105	EAHPASQGKVNGDTCLTLSAMGI</																					

Table 2: Nucleotide and polypeptide sequences of additional OX2R homologs.

primate, e.g., human, OX2RH1 (SEQ ID NO: 3 and 4):

5	cagagaaaag cttctgttcg tccaagttac taaccaggct aaaccacata gacgtgaagg	60
	aaggggctag aaggaaggga gtgccccact gttgatgggg taagaggatc ctgtactgag	120
10	aagttgacca gagaggggtct caccatgcgc acagttcctt ctgtaccagt gtggaggaaa	180
	agtactgagt gaagggcaga aaaagagaaa acagaa atg ctc tgc cct tgg aga	234
		Met Leu Cys Pro Trp Arg
		-25
15	act gct aac cta ggg cta ctg ttg att ttg act atc ttc tta gtg gcc	282
	Thr Ala Asn Leu Gly Leu Leu Ile Leu Thr Ile Phe Leu Val Ala	
	-20 -15 -10 -5	
20	gaa gcg gag ggt gct gct caa cca aac aac tca tta atg ctg caa act	330
	Glu Ala Glu Gly Ala Ala Gln Pro Asn Asn Ser Leu Met Leu Gln Thr	
	-1 1 5 10	
25	agc aag gag aat cat gct tta gct tca agc agt tta tgt atg gat gaa	378
	Ser Lys Glu Asn His Ala Leu Ala Ser Ser Ser Leu Cys Met Asp Glu	
	15 20 25	
30	aaa cag att aca cag aac tac tcg aaa gta ctc gca gaa gtt aac act	426
	Lys Gln Ile Thr Gln Asn Tyr Ser Lys Val Leu Ala Glu Val Asn Thr	
	30 35 40	
35	tca tgg cct gta aag atg gct aca aat gct gtg ctt tgt tgc cct cct	474
	Ser Trp Pro Val Lys Met Ala Thr Asn Ala Val Leu Cys Cys Pro Pro	
	45 50 55 60	
40	atc gca tta aga aat ttg atc ata ata aca tgg gaa ata atc ctg aga	522
	Ile Ala Leu Arg Asn Leu Ile Ile Ile Thr Trp Glu Ile Ile Leu Arg	
	65 70 75	
45	ggc cag cct tcc tgc aca aaa gcc tac aag aaa gaa aca aat gag acc	570
	Gly Gln Pro Ser Cys Thr Lys Ala Tyr Lys Lys Glu Thr Asn Glu Thr	
	80 85 90	
50	aag gaa acc aac tgt act gat gag aga ata acc tgg gtc tcc aga cct	618
	Lys Glu Thr Asn Cys Thr Asp Glu Arg Ile Thr Trp Val Ser Arg Pro	
	95 100 105	
55	gat cag aat tcg gac ctt cag att cgt acc gtg gcc atc act cat gac	666
	Asp Gln Asn Ser Asp Leu Gln Ile Arg Thr Val Ala Ile Thr His Asp	
	110 115 120	
60	ggg tat tac aga tgc ata atg gta aca cct gat ggg aat ttc cat cgt	714
	Gly Tyr Tyr Arg Cys Ile Met Val Thr Pro Asp Gly Asn Phe His Arg	
	125 130 135 140	
65	gga tat cac ctc caa gtg tta gtt aca cct gaa gtg acc ctg ttt caa	762
	Gly Tyr His Leu Gln Val Leu Val Thr Pro Glu Val Thr Leu Phe Gln	
	145 150 155	

aac agg aat aga act gca gta tgc aag gca gtt gca ggg aag cca gct 810
 Asn Arg Asn Arg Thr Ala Val Cys Lys Ala Val Ala Gly Lys Pro Ala
 160 165 170

5 gcg cat atc tcc tgg atc cca gag ggc gat tgt gcc act aag caa gaa 858
 Ala His Ile Ser Trp Ile Pro Glu Gly Asp Cys Ala Thr Lys Gln Glu
 175 180 185

10 tac tgg agc aat ggc aca gtg act gtt aag agt aca tgc cac tgg gag 906
 Tyr Trp Ser Asn Gly Thr Val Thr Val Lys Ser Thr Cys His Trp Glu
 190 195 200

15 gtc cac aat gtg tct acc gtg acc tgc cac gtc tcc cat ttg act ggc 954
 Val His Asn Val Ser Thr Val Thr Cys His Val Ser His Leu Thr Gly
 205 210 215 220

20 aac aag agt ctg tac ata gag cta ctt cct gtt cca ggt gcc aaa aaa 1002
 Asn Lys Ser Leu Tyr Ile Glu Leu Leu Pro Val Pro Gly Ala Lys Lys
 225 230 235

25 atc agc aaa att ata tat tcc ata tat cat cct tac tat tat tat tta 1050
 Ile Ser Lys Ile Ile Tyr Ser Ile Tyr His Pro Tyr Tyr Tyr Tyr Leu
 240 245 250

30 gac cat cgt ggg att cat ttg gtt gtt gaa agt caa tgg ctg cag aaa 1098
 Asp His Arg Gly Ile His Leu Val Val Glu Ser Gln Trp Leu Gln Lys
 255 260 265

35 ata taaattgaat aaaacagaat ctactccagt tggtgaggag gatgaaatgc 1151
 Ile

40 agccctatgc cagctacaca gagaagaaca atcctctcta tgatactaca aacaagggtga 1211

45 aggcattctga ggcattacaa agtgaagttg acacagacct ccatacttta taagttggtg 1271

50 gactctagta ccaagaaaca acaacaaacg agatacatta taattactgt ctgattttct 1331

55 tacagttcta gaatgaagac ttatattgaa attaggtttt ccaaggttct tagaagacat 1391

60 tttaatggat tctcattcat acccttgtat aattggaatt tttgattctt agctgctacc 1451

65 agctagttct ctgaagaact gatgttatta caaagaaaat acatgcccac gaccaaatat 1511

70 tcaaattgtg caggacagta aataatgaaa accaaatttc ctcaagaaat aactgaagaa 1571

75 ggagcaagtg tgaacagttt cttgtgtatc ctt 1604

80 MLCPWRTANLGLLLILTIFLVAEAGAAQPNNSLMLQTSKENHALASSSLCMDEKQITONYSKVLAEVNTSWPVKM
 ATNAVLCCPPIALRNLIITWEIILRGQPSCTKAYKETNETKETNCTDERITWVSRPDQNSDLQIRTVAITHDGY
 YRCIMVTPDGNFHRGYHLQVLVTPFVTLFQNRNRTAVCKAVAGKPAAHISWIPEGDCATKQEYWSNGTVTVKSTCH
 WEVHNVTSTVCHVSHLTGNKSLYIELLPVPGAKKISKIYSIYHPYYYYLDHRGIHLVVESQWLQKI

primate, e.g., human, OX2RH1.2 (SEQ ID NO: 19 and 20):

5	atg ctc tgc cct tgg aga act gct aac cta ggg cta ctg ttg att ttg	48
	Met Leu Cys Pro Trp Arg Thr Ala Asn Leu Gly Leu Leu Leu Ile Leu	
	-25 -20 -15	
10	act atc ttc tta gtg gcc gaa gcg gag ggt gct gct caa cca aac aac	96
	Thr Ile Phe Leu Val Ala Glu Ala Glu Gly Ala Ala Gln Pro Asn Asn	
	-10 -5 -1 1 5	
15	tca tta atg ctg caa act agc aag gag aat cat gct tta gct tca agc	144
	Ser Leu Met Leu Gln Thr Ser Lys Glu Asn His Ala Leu Ala Ser Ser	
	10 15 20	
20	agt tta tgt atg gat gaa aaa cag att aca cag aac tac tcg aaa gta	192
	Ser Leu Cys Met Asp Glu Lys Gln Ile Thr Gln Asn Tyr Ser Lys Val	
	25 30 35	
25	ctc gca gaa gtt aac act tca tgg cct gta aag atg gct aca aat gct	240
	Leu Ala Glu Val Asn Thr Ser Trp Pro Val Lys Met Ala Thr Asn Ala	
	40 45 50	
30	gtg ctt tgt tgc cct cct atc gca tta aga aat ttg atc ata ata aca	288
	Val Leu Cys Cys Pro Pro Ile Ala Leu Arg Asn Leu Ile Ile Ile Thr	
	55 60 65 70	
35	tgg gaa ata atc ctg aga ggc cag cct tcc tgc aca aaa gcc tac agg	336
	Trp Glu Ile Ile Leu Arg Gly Gln Pro Ser Cys Thr Lys Ala Tyr Arg	
	75 80 85	
40	aaa gaa aca aat gag acc aag gaa acc aac tgt act gat gag aga ata	384
	Lys Glu Thr Asn Glu Thr Lys Glu Thr Asn Cys Thr Asp Glu Arg Ile	
	90 95 100	
45	acc tgg gtc tcc aga cct gat cag aat tcg gac ctt cag att cgt cca	432
	Thr Trp Val Ser Arg Pro Asp Gln Asn Ser Asp Leu Gln Ile Arg Pro	
	105 110 115	
50	gtg gcc atc act cat gac ggg tat tac aga tgc ata atg gta aca cct	480
	Val Ala Ile Thr His Asp Gly Tyr Tyr Arg Cys Ile Met Val Thr Pro	
	120 125 130	
55	gat ggg aat ttc cat cgt gga tat cac ctc caa gtg tta gtt aca cct	528
	Asp Gly Asn Phe His Arg Gly Tyr His Leu Gln Val Leu Val Thr Pro	
	135 140 145 150	
60	gaa gtg acc ctg ttt caa aac agg aat aga act gca gta tgc aag gca	576
	Glu Val Thr Leu Phe Gln Asn Arg Asn Arg Thr Ala Val Cys Lys Ala	
	155 160 165	
65	gtt gca ggg aag cca gct gcg cag atc tcc tgg atc cca gag ggc gat	624
	Val Ala Gly Lys Pro Ala Ala Gln Ile Ser Trp Ile Pro Glu Gly Asp	
	170 175 180	
70	tgt gcc act aag caa gaa tac tgg agc aat ggc aca gtg act gtt aag	672
	Cys Ala Thr Lys Gln Glu Tyr Trp Ser Asn Gly Thr Val Thr Val Lys	
	185 190 195	

	agt	aca	tgc	cac	tgg	gag	gtc	cac	aat	gtg	tct	acc	gtg	acc	tgc	cac	720
	Ser	Thr	Cys	His	Trp	Glu	Val	His	Asn	Val	Ser	Thr	Val	Thr	Cys	His	
	200					205					210						
5	gtc	tcc	cat	ttg	act	ggc	aac	aag	agt	ctg	tac	ata	gag	cta	ctt	cct	768
	Val	Ser	His	Leu	Thr	Gly	Asn	Lys	Ser	Leu	Tyr	Ile	Glu	Leu	Leu	Pro	
	215					220					225					230	
10	gtt	cca	ggt	gcc	aaa	aaa	tca	gca	aaa	tta	tat	att	cca	tat	atc	atc	816
	Val	Pro	Gly	Ala	Lys	Lys	Ser	Ala	Lys	Leu	Tyr	Ile	Pro	Tyr	Ile	Ile	
						235					240					245	
15	ctt	act	att	att	att	ttg	acc	atc	gtg	gga	ttc	att	tgg	ttg	ttg	aaa	864
	Leu	Thr	Ile	Ile	Ile	Leu	Thr	Ile	Val	Gly	Phe	Ile	Trp	Leu	Leu	Lys	
						250					255					260	
20	gtc	aat	ggc	tgc	aga	aaa	tat	aaa	ttg	aat	aaa	aca	gaa	tct	act	cca	912
	Val	Asn	Gly	Cys	Arg	Lys	Tyr	Lys	Leu	Asn	Lys	Thr	Glu	Ser	Thr	Pro	
	265					270					275						
25	gtt	gtt	gag	gag	gat	gaa	atg	cag	ccc	tat	gcc	agc	tac	aca	gag	aag	960
	Val	Val	Glu	Glu	Asp	Glu	Met	Gln	Pro	Tyr	Ala	Ser	Tyr	Thr	Glu	Lys	
	280					285					290						
30	aac	aat	cct	ctc	tat	gat	act	aca	aac	aag	gtg	aag	gca	tct	cag	gca	1008
	Asn	Asn	Pro	Leu	Tyr	Asp	Thr	Thr	Asn	Lys	Val	Lys	Ala	Ser	Gln	Ala	
	295					300					305					310	
35	tta	caa	agt	gaa	gtt	gac	aca	gac	ctc	cat	act	tta	taa				1047
	Leu	Gln	Ser	Glu	Val	Asp	Thr	Asp	Leu	His	Thr	Leu					
						315					320						
40	MLCPWRTANLGLLLILTIFLVAEAEAGAAQPNNSLMLQTSKENHALASSSLCMDEKQITQNYSKVLAEVNTSWPVKM ATNAVLCCPPIALRNLIITWEIILRGQPSTCKAYRKETNETKETNCTDERITWVSRPDQNSDLQIRPVAITHDGY YRCIMVTDPDGNFHRGYHLQVLVTP EVT L F Q N R N R T A V C K A V A G K P A A Q I S W I P E G D C A T K Q E Y W S N G T V T V K S T C H WEVHNVTSTVCHVSHLTGKNKSLYIELLPVPGAKKSAKLYIPYIIILTIIVGFIIWLLKVNGCRKYKLNKTESTP VVEEDEMQPYASYTEKNPNPLYDTTNKVKAQALQSEVDTDLHTLZ																

rodent, e.g., mouse, OX2RH1 (SEQ ID NO: 5 and 6):

```

5   aaaaccgaa atg ttt tgc ttt tgg aga act tct gcc cta gca gtg ctc tta 51
    Met Phe Cys Phe Trp Arg Thr Ser Ala Leu Ala Val Leu Leu
    1           5           10

10  ata tgg ggg gtc ttt gtg gct ggg tca agt tgt act gat aag aat caa 99
    Ile Trp Gly Val Phe Val Ala Gly Ser Ser Cys Thr Asp Lys Asn Gln
    15           20           25           30

15  aca aca cag aac aac agt tca tct cct ctg aca caa gtg aac act aca 147
    Thr Thr Gln Asn Asn Ser Ser Ser Pro Leu Thr Gln Val Asn Thr Thr
    35           40           45

20  gtg tct gta cag ata ggt aca aag gct ctg ctc tgc tgc ttt tct att 195
    Val Ser Val Gln Ile Gly Thr Lys Ala Leu Leu Cys Cys Phe Ser Ile
    50           55           60

25  cca ctg aca aaa gca gta tta atc aca tgg ata ata aag ctc aga ggc 243
    Pro Leu Thr Lys Ala Val Leu Ile Thr Trp Ile Ile Lys Leu Arg Gly
    65           70           75

30  ctg cca tcc tgc aca ata gca tac aaa gta gat aca aag acc aat gaa 291
    Leu Pro Ser Cys Thr Ile Ala Tyr Lys Val Asp Thr Lys Thr Asn Glu
    80           85           90

35  acc agc tgc ttg ggc agg aac atc acc tgg gcc tcc aca cct gac cac 339
    Thr Ser Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro Asp His
    95           100          105          110

40  agt cct gaa ctt cag atc agt gca gtg acc ctc cag cat gag ggg act 387
    Ser Pro Glu Leu Gln Ile Ser Ala Val Thr Leu Gln His Glu Gly Thr
    115          120          125

45  tac aca tgt gag aca gta aca cct gaa ggg aat ttt gaa aaa aac tat 435
    Tyr Thr Cys Glu Thr Val Thr Pro Glu Gly Asn Phe Glu Lys Asn Tyr
    130          135          140

50  gac ctc caa gtg ctg gtg ccc cct gaa gta acc tac ttt cca gag aaa 483
    Asp Leu Gln Val Leu Val Pro Pro Glu Val Thr Tyr Phe Pro Glu Lys
    145          150          155

55  aac aga tct gca gtc tgt gag gca atg gca ggc aag cct gct gca cag 531
    Asn Arg Ser Ala Val Cys Glu Ala Met Ala Gly Lys Pro Ala Ala Gln
    160          165          170

60  atc tct tgg tct cca gat ggg gac tgt gtc act acg agt gaa tca cac 579
    Ile Ser Trp Ser Pro Asp Gly Asp Cys Val Thr Thr Ser Glu Ser His
    175          180          185          190

65  agc aat ggc act gtg act gtc agg agc aca tgc cac tgg gag cag aac 627
    Ser Asn Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu Gln Asn
    195          200          205

70  aat gtg tct gat gtg tcc tgc att gtc tct cat ttg act ggt aac caa 675
    Asn Val Ser Asp Val Ser Cys Ile Val Ser His Leu Thr Gly Asn Gln
    210          215          220

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tct ctg tcc ata gaa ctg agt aga ggt ggt aac caa tca tta cga cca 723
 Ser Leu Ser Ile Glu Leu Ser Arg Gly Gly Asn Gln Ser Leu Arg Pro
 225 230 235

5 tat att cca tac atc ata cca tca att atc att ttg atc atc ata gga 771
 Tyr Ile Pro Tyr Ile Ile Pro Ser Ile Ile Ile Leu Ile Ile Ile Gly
 240 245 250

10 tgc att tgt ctt ttg aaa atc agt ggc ttc aga aaa tgc aaa ttg cca 819
 Cys Ile Cys Leu Leu Lys Ile Ser Gly Phe Arg Lys Cys Lys Leu Pro
 255 260 265 270

15 aaa tta gaa gct act tca gct att gag gag gat gaa atg cag cct tat 867
 Lys Leu Glu Ala Thr Ser Ala Ile Glu Glu Asp Glu Met Gln Pro Tyr
 275 280 285

20 gct agc tat aca gag aag agc aat cca ctc tat gat act gtg act aag 915
 Ala Ser Tyr Thr Glu Lys Ser Asn Pro Leu Tyr Asp Thr Val Thr Lys
 290 295 300

gtg gag gca ttt cca gta tca caa ggc gaa gtc aat ggc aca gac tgc 963
 Val Glu Ala Phe Pro Val Ser Gln Gly Glu Val Asn Gly Thr Asp Cys
 305 310 315

25 ctt act ttg tcg gcc att gga atc tagaaccaag aaaaaagaag tcaagagaca 1017
 Leu Thr Leu Ser Ala Ile Gly Ile
 320 325

30 tcataattac tgctttgctt tctttaaaat tcgacaatgg aaggactact tggaaattag 1077
 ctcttccaaa gctattataaaa agcacaaatg ttctaatagaa attgcattta aattctatca 1137
 ttggaagttt ggaatctctg ctgctacctg ttaatttttag gaagaactga ttttaattatt 1197

35 acaaagaaag cacatgggta tgggtgaaata tcaagttgtg caataaagta tgatgaaaac 1257
 tgagtttcct caagaaataa ctgcaggagg aacaatcatc actaaagaat ttcattgtgag 1317
 ttcttacaaa aaaattccta tgtatacatg actatgggtat gtgtgtccaa ttacatgttt 1377

40 atttacaaat gtgtatatat gcacacattt gcttttcagg acatctcctt gtaaaaaaca 1437
 cactggagtt ttggatttat aaaagcttat aaagtgagca ttggagatat ttt 1490

45 MFCFWRTSALAVLLIWGVFVAGSSCTDKNQTTQNNSSSPLTQVNTTVSVQIGTKALLCCFSIPLTKAVLITWIIKL
 RGLPSCTIAYKVDTKNETSCLGRNITWASTPDHSPQLISAVTLQHEGTYTCETVTPEGNFEEKNYDLQVLVPEV
 TYFPEKNRSVCEAMAGKPAAQISWSPDGDCVTTSESHSNGTVTVRSTCHWEQNNVSDVSCIVSHLTGNQSLSEL
 SRGGNQSLRPYIPYIIPSIILIIIGCICLLKISGFRKCKLPKLEATSAIEEDEMOPYASYTEKSNPLYDVTVE
 50 AFPVSQGEVNGTDCLTSLAIGI

5	atg Met	ggt Gly	gga Gly	aag Lys	cag Gln	atg Met	aca Thr	cag Gln	aac Asn	tat Tyr	tca Ser	aca Thr	att Ile	ttt Phe	gca Ala	gaa Glu	48
	1				5					10					15		
10	ggt Gly	aac Asn	att Ile	tca Ser	cag Gln	cct Pro	gta Val	ctg Leu	atg Met	gat Asp	ata Ile	aat Asn	gct Ala	gtg Val	ctt Leu	tgt Cys	96
				20					25					30			
15	tgc Cys	cct Pro	cct Pro	att Ile	gca Ala	tta Leu	aga Arg	aat Asn	ttg Leu	atc Ile	ata Ile	ata Ile	aca Thr	tgg Trp	gaa Glu	ata Ile	144
				35				40					45				
20	atc Ile	ctg Leu	aga Arg	ggc Gly	cag Gln	cct Pro	tcc Ser	tgc Cys	aca Thr	aaa Lys	gcc Ala	tac Tyr	aag Lys	aaa Lys	gaa Glu	aca Thr	192
		50					55					60					
25	aat Asn	gag Glu	acc Thr	aag Lys	gaa Glu	acc Thr	aac Asn	tgt Cys	act Thr	gtt Val	gag Glu	aga Arg	ata Ile	acc Thr	tgg Trp	gtc Val	240
	65					70					75					80	
30	tct Ser	aga Arg	cct Pro	gat Asp	cag Gln	aat Asn	tcg Ser	gac Asp	ctt Leu	cag Gln	att Ile	cgt Arg	ccg Pro	gtg Val	gac Asp	acc Thr	288
					85					90					95		
35	act Thr	cat His	gac Asp	ggg Gly	tat Tyr	tac Tyr	aga Arg	ggc Gly	ata Ile	gtg Val	gta Val	aca Thr	cct Pro	gat Asp	ggg Gly	aat Asn	336
				100				105						110			
40	ttc Phe	cat His	cgt Arg	gga Gly	tat Tyr	cac His	ctc Leu	caa Gln	gtg Val	tta Leu	gtt Val	aca Thr	ccc Pro	gaa Glu	gtg Val	aac Asn	384
			115					120					125				
45	cta Leu	ttt Phe	caa Gln	agc Ser	agg Arg	aat Asn	ata Ile	act Thr	gca Ala	gta Val	tgc Cys	aag Lys	gca Ala	gtt Val	aca Thr	ggg Gly	432
		130					135					140					
50	aag Lys	cca Pro	gct Ala	gcc Ala	cag Gln	atc Ile	tcc Ser	tgg Trp	atc Ile	cca Pro	gag Glu	gga Gly	tct Ser	att Ile	ctt Leu	gcc Ala	480
	145					150					155					160	
55	act Thr	aag Lys	caa Gln	gaa Glu	tac Tyr	tgg Trp	ggc Gly	aat Asn	ggc Gly	aca Thr	gtg Val	acg Thr	gtt Val	aag Lys	agt Ser	aca Thr	528
					165					170					175		
60	tgc Cys	ccc Pro	tgg Trp	gag Glu	ggc Gly	cac His	aag Lys	tct Ser	act Thr	gtg Val	acc Thr	tgc Cys	cat His	gtc Val	tcc Ser	cat His	576
				180					185					190			
65	ttg Leu	act Thr	ggc Gly	aac Asn	aag Lys	agt Ser	ctg Leu	tcc Ser	gta Val	aag Lys	ttg Leu	aat Asn	tca Ser	ggg Gly	ctc Leu	aga Arg	624
			195					200					205				
70	acc Thr	tca Ser	gga Gly	tct Ser	cca Pro	gcg Ala	ttg Leu	tcc Ser	tta Leu	ctg Leu	atc Ile	att Ile	ctt Leu	tat Tyr	gtg Val	aaa Lys	672
		210				215						220					

cag aac aat gtg tct gct gtg tcc tgc att gtc tct cat tcg act ggt 432
Gln Asn Asn Val Ser Ala Val Ser Cys Ile Val Ser His Ser Thr Gly
130 135 140

	aat	cag	tct	ctg	tcc	ata	gaa	ctg	agt	aga	ggg	acc	acc	agc	acc	acc	480	
	Asn	Gln	Ser	Leu	Ser	Ile	Glu	Leu	Ser	Arg	Gly	Thr	Thr	Ser	Thr	Thr		
5	145					150					155					160		
	cct	tcc	ttg	ctg	acc	att	ctc	tac	gtg	aaa	atg	gtc	ctt	ttg	ggg	att	528	
	Pro	Ser	Leu	Leu	Thr	Ile	Leu	Tyr	Val	Lys	Met	Val	Leu	Leu	Gly	Ile		
					165					170					175			
10	att	ctt	ctt	aaa	gtg	gga	ttt	gct	ttc	ttc	cag	aag	aga	aat	gtt	acc	576	
	Ile	Leu	Leu	Lys	Val	Gly	Phe	Ala	Phe	Phe	Gln	Lys	Arg	Asn	Val	Thr		
				180					185					190				
15	aga	aca	tga	at	atcca	gatttct	tgga	agctc	attag	tctgat	gaca	cat	accagaa				632	
	Arg	Thr																
	aac	agc	attt	gta	atca	act	ttctc	attgg	aatcc	agctt	acccg	tccct	gctgt	cttca			692	
20	tg	tttg	tttag	ac	actcac	ct	ccaa	attctt	aactg	agaag	ggctc	ctgtc	t	aaagg	aaat		752	
	at	gggg	gacaa	attgt	ggagc	atag	ac	caaaa	agaa	aggcca	tccag	agact	gcccc	accta			812	
	agg	accc	catc	ccat	ata	acag	acac	caaacc	cagac	actac	tga	agat	gct	gcga	agcg	tt	872	
25	tg	ctg	ac	agg	agc	ctgt	t	at	agctgt	ctcc	tgag	ag	gctc	agccag	agcc	tgac	aaatac	932
	atag	gtag	at	gctt	gcag	cc	aaca	actgga	ctgag	caaaa	aatct	ccatt	ggagg	ag	tta		992	
30	gag	aaagg	gac	tga	agagg	gt	gaa	agggtt	gcag	ccccat	agga	agaaca	aca	at	atcaa		1052	
	cca	accag	at	ctccc	agagc	tccc	agg	ggac	taa								1085	
35	RGQPSCIMAYKVETKETNETCLGRNITWASTPDHIPDLQISAVALQHEGNYLCEITTPEGNFHKVYDLQVLVPPPEV TYFLGENRTAVCEAMAGKPAAQISWTPDGDVCVKSESHSNGTVTVRSTCHWEQNNVSAVSCIVSHSTGNQSLSIEL SRGTTSTTPSLLTILYVKMVLGLIILLKVGFAFFQKRNVTRT																	
40	Rodent, e.g., mouse, OX2RH4 (SEQ ID NO: 22 and 23):																	
	atg	cat	gct	ctg	ggg	agg	att	ccg	act	ttg	act	ttg	ctg	atc	ttc	atc	48	
	Met	His	Ala	Leu	Gly	Arg	Ile	Pro	Thr	Leu	Thr	Leu	Leu	Ile	Phe	Ile		
	-25					-20				-15						-10		
45	aat	att	ttt	gtg	tct	ggg	tca	agt	tgt	act	gat	gag	aat	caa	aca	ata	96	
	Asn	Ile	Phe	Val	Ser	Gly	Ser	Ser	Cys	Thr	Asp	Glu	Asn	Gln	Thr	Ile		
					-5				-1	1				5				
50	cag	aat	gac	agt	tca	tct	tct	ctg	aca	caa	gtt	aac	act	aca	atg	tct	144	
	Gln	Asn	Asp	Ser	Ser	Ser	Ser											

	tcc tgc aca ata gca tac aac cta gat aaa aag acc aat gaa acc agc	288
	Ser Cys Thr Ile Ala Tyr Asn Leu Asp Lys Lys Thr Asn Glu Thr Ser	
	60 65 70	
5	tgc ttg ggc agg aac atc acc tgg gcc tcc aca cct gac cac agt cct	336
	Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro Asp His Ser Pro	
	75 80 85	
10	gaa ctt cag atc agt gca gtg gcc ctc cag cat gag ggg act tac aca	384
	Glu Leu Gln Ile Ser Ala Val Ala Leu Gln His Glu Gly Thr Tyr Thr	
	90 95 100	
15	tgt gag ata gta aca cct gaa ggg aat tta gaa aaa gtc tat gac ctc	432
	Cys Glu Ile Val Thr Pro Glu Gly Asn Leu Glu Lys Val Tyr Asp Leu	
	105 110 115	
20	caa gtg ctg gtg ccc cct gag gta acc tac ttt cca ggg aaa aac aga	480
	Gln Val Leu Val Pro Pro Glu Val Thr Tyr Phe Pro Gly Lys Asn Arg	
	120 125 130 135	
25	act gca gtc tgt gag gca atg gca ggc aag cct gct gca cag atc tct	528
	Thr Ala Val Cys Glu Ala Met Ala Gly Lys Pro Ala Ala Gln Ile Ser	
	140 145 150	
30	tgg act cca gat ggg gac tgt gtc act aag agt gag tca cac agc aat	576
	Trp Thr Pro Asp Gly Asp Cys Val Thr Lys Ser Glu Ser His Ser Asn	
	155 160 165	
35	ggc act gtg act gtc agg agc acg tgc cac tgg gag cag aac aat gtg	624
	Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu Gln Asn Asn Val	
	170 175 180	
40	tct gtt gtg tcc tgc tta gtc tct cat tcg act ggt aat cag tct ctg	672
	Ser Val Val Ser Cys Leu Val Ser His Ser Thr Gly Asn Gln Ser Leu	
	185 190 195	
45	tcc ata gaa ctg agt caa ggt aca atg acc acc ccc cgt tcc ttg ctg	720
	Ser Ile Glu Leu Ser Gln Gly Thr Met Thr Thr Pro Arg Ser Leu Leu	
	200 205 210 215	
50	acc att ctc tat gtg aaa atg gcc ctt ttg gtg att att ctt ctt aac	768
	Thr Ile Leu Tyr Val Lys Met Ala Leu Leu Val Ile Ile Leu Leu Asn	
	220 225 230	
55	gta gga ttt gct ttc ttc cag aag aga aat ttt gcc aga aca tga	813
	Val Gly Phe Ala Phe Phe Gln Lys Arg Asn Phe Ala Arg Thr	
	235 240 245	
60	MHALGRIPTLLIFINIFVSGSSCTDENQTIQNDSSSSLTQVNTTMSVQMDKKALLCCFSSPLINAVLIT	
	WIIKHRHLPSTCIAYNLDKKTNETSCLGRNITWASTPDHSPQLQISAVLQHEGTYTCEIVTPEGNLEKVY	
	DLQVLVPPEVTYFPGKNRTAVCEAMAGKPAAQISWTPDGDVTKSEHSNGTVTVRSTCHWEQNNVSVVSC	
	LVSHSTGNQSLSIELSQGTMTTPRSLLTILYVKMALLVIILLNVGFAFFQKRNFART	

Table 3: Rodent, e.g., mouse, OX2RH3 (SEQ ID NO: 11 and 12):

	ggcacgagtt acgattttgtg cttaacctga ctccactcca g atg cat gct ttg ggg 56																
	Met His Ala Leu Gly																
5	-25																
	agg	act	ctg	gct	ttg	atg	tta	ctc	atc	ttc	atc	act	att	ttg	gtg	cct	104
	Arg	Thr	Leu	Ala	Leu	Met	Leu	Leu	Ile	Phe	Ile	Thr	Ile	Leu	Val	Pro	
	-20					-15					-10					-5	
10																	
	gag	tca	agt	tgt	tca	gtg	aaa	gga	cgg	gag	gag	atc	cca	ccg	gat	gat	152
	Glu	Ser	Ser	Cys	Ser	Val	Lys	Gly	Arg	Glu	Glu	Ile	Pro	Pro	Asp	Asp	
				-1	1				5					10			
15																	
	tca	ttt	cct	ttt	tca	gat	gat	aat	atc	ttc	cct	gat	gga	gtg	ggc	gtc	200
	Ser	Phe	Pro	Phe	Ser	Asp	Asp	Asn	Ile	Phe	Pro	Asp	Gly	Val	Gly	Val	
			15					20					25				
20																	
	acc	atg	gag	att	gag	att	atc	act	cca	gtg	tct	gta	cag	ata	ggc	atc	248
	Thr	Met	Glu	Ile	Glu	Ile	Ile	Thr	Pro	Val	Ser	Val	Gln	Ile	Gly	Ile	
		30					35					40					
25																	
	aag	gct	cag	ctt	ttc	tgt	cat	cct	agt	cca	tca	aaa	gaa	gca	aca	ctt	296
	Lys	Ala	Gln	Leu	Phe	Cys	His	Pro	Ser	Pro	Ser	Lys	Glu	Ala	Thr	Leu	
	45					50					55					60	
30																	
	aga	ata	tggt	gaa	ata	act	ccc	aga	gac	tggt	cct	tcc	tgc	aga	cta	ccc	344
	Arg	Ile	Trp	Glu	Ile	Thr	Pro	Arg	Asp	Trp	Pro	Ser	Cys	Arg	Leu	Pro	
					65					70					75		
35																	
	tac	aga	gca	gag	ttg	cag	cag	atc	agt	aaa	aaa	atc	tgt	act	gag	aga	392
	Tyr	Arg	Ala	Glu	Leu	Gln	Gln	Ile	Ser	Lys	Lys	Ile	Cys	Thr	Glu	Arg	
				80					85					90			
40																	
	gga	acc	act	agg	gtc	cct	gca	cat	cac	cag	agt	tct	gac	ctt	ccc	atc	440
	Gly	Thr	Thr	Arg	Val	Pro	Ala	His	His	Gln	Ser	Ser	Asp	Leu	Pro	Ile	
			95					100					105				
45																	
	aaa	tca	atg	gcc	ctc	aag	cat	gat	ggg	cat	tac	tca	tgt	cgg	ata	gaa	488
	Lys	Ser	Met	Ala	Leu	Lys	His	Asp	Gly	His	Tyr	Ser	Cys	Arg	Ile	Glu	
	110						115					120					
50																	
	aca	aca	gat	ggg	att	ttc	caa	gag	aga	cat	agc	atc	caa	gtg	cca	ggg	536
	Thr	Thr	Asp	Gly	Ile	Phe	Gln	Glu	Arg	His	Ser	Ile	Gln	Val	Pro	Gly	
	125					130					135					140	
55																	
	gaa	aat	aga	act	gta	gtt	tgt	gag	gca	att	gca	agc	aag	cct	gct	atg	584
	Glu	Asn	Arg	Thr	Val	Val	Cys	Glu	Ala	Ile	Ala	Ser	Lys	Pro	Ala	Met	
					145					150					155		
60																	
	cag	atc	ttg	tggt	act	cca	gat	gag	gac	tgt	gtc	act	aag	agt	aaa	tca	632
	Gln	Ile	Leu	Trp	Thr	Pro	Asp	Glu	Asp	Cys	Val	Thr	Lys	Ser	Lys	Ser	
				160					165					170			
65																	
	cac	aat	gac	acc	atg	att	gtc	agg	agc	aag	tgc	cac	agg	gag	aaa	aac	680
	His	Asn	Asp	Thr	Met	Ile	Val	Arg	Ser	Lys	Cys	His	Arg	Glu	Lys	Asn	
			175					180					185				

34

aat ggc cac agt gtg ttc tgc ttt atc tcc cat ttg act gat aac tgg 728
 Asn Gly His Ser Val Phe Cys Phe Ile Ser His Leu Thr Asp Asn Trp
 190 195 200

5 att ctc tcc atg gaa cag aat cga ggt aca acc agc atc ctg cct tcc 776
 Ile Leu Ser Met Glu Gln Asn Arg Gly Thr Thr Ser Ile Leu Pro Ser
 205 210 215 220

10 ttg ctg agc att ctc tat gtg aaa ctg gct gta act gtt ctc atc gta 824
 Leu Leu Ser Ile Leu Tyr Val Lys Leu Ala Val Thr Val Leu Ile Val
 225 230 235

15 gga ttt gct ttt ttc cag aag aga aat tat ttc aga gtg cca gaa ggc 872
 Gly Phe Ala Phe Phe Gln Lys Arg Asn Tyr Phe Arg Val Pro Glu Gly
 240 245 250

tcc tgaggagagt ggtctgtggt taagatgaga ttaccacca tctgaaagac 925
 Ser

20 atcttgtcta ccgcgcagcg tgctgagatt ccgagaagca gccacagaac ctactaggaa 985
 gacaaatctg atgtggttgt caatcctttc aatggacctg agtacttcta taaacccgag 1045

25 tgaggttgtg ctggaccag gagccaggct aggtcatata tgttgatttt tgctgcaaga 1105
 cctcatgggtt tatctacaaa tcctaaattc tttcacttcc agtttttaaaa cttttggccc 1165
 aagcattttta tccacagcat aacaccttta aagaaactct cccacggaaa ctgctggttc 1225

30 catggaatgg aaaattgcaa catgggtttac aagacagtgc aaaccaagca gcattccaag 1285
 atatgagctt cagaaagtta caggaactgt cttgggacga gaaagaagga ttaaatagtt 1345

35 cccagtccc 1354

40 MHALGRTLALMLLIFITILVPESSCSVKGREEIPPDDSFPPSDDNIFPDGVGVMTMEIEIITPVSVQIGIKAQLFCH
 PPSKEATLRIWEITPRDWPSCLPYRAELQQISKKICTERGTTTRVPAHHQSSDLPIKSMALKHGDGHYSCRIETTD
 GIFQERHSIQVPGENRTVVCEAIASKPAMQILWTPDEDCVTKSKSHNDTMIVRSKCHREKNNGHSVFCFISHLTDN
 WILSMEQNRGTTSILPSLLSILYVKLAVTVLIVGFAFFQKRNYFRVPEGS

Table 4: Reverse translations of OX2R homologs:

rodent, e.g., rat, OX2RH1 (SEQ ID NO: 13):

5	atgytntgyt	tytggmgna	c nwsncaygtn	gcngtnytny	tnathtgggg	ngtnttygcn	60
	gcngarwsnw	sntgyccnga	yaaraaycar	acnatgcara	ayaaywsnws	nacnatgacn	120
	gargtnaaya	cnacngtntt	ygtncaratg	ggnaaraarg	cnytnytntg	ytgyccnwsn	180
	athwsnytna	cnaargtnat	hytnathacn	tggacnatha	cnytnmgngg	ncarccnwsn	240
	tgyathathw	sntayaargc	ngayacnmgn	garacncayg	arwsnaaytg	ywsngaymgn	300
10	wsnathacnt	gggcnwsnac	nccngayytn	gcncngayy	tncarathtws	ngcngtngcn	360
	ytncarcayg	arggnmgnta	ywsntgygay	athgcngtnc	cngayggnaa	yttycaraay	420
	athtaygayy	tncargtnyt	ngtnccnccn	gargtnacnc	ayttyccngg	ngaraaymgn	480
	acngcngtnt	gygargcnat	hgcnngnaar	ccngcngcnc	arathwsntg	gacnccngay	540
	ggngaytggy	tngcnaaraa	ygarwsncay	wsnaayggna	cngtnacngt	nmgnwsnacb	600
15	tgycaytggy	arcarwsnca	ygtnwsngtn	gtnttytggy	tngtnwsnca	yytnacnacn	660
	ggnaaycarw	snytnwsnat	hgarytnggn	mgngggngng	aycarytnyt	nggnwsntay	720
	athcartaya	thathccnws	nathathath	ytathathath	thggntgyat	htgyytnytn	780
	aarathwsng	gntgymgnaa	rtgyaarytn	ccnaarwsng	gngcnacncc	ngayathgar	840
	gargaygara	tgcarcanta	ygcnwsntay	acngaraarw	snaayccnyt	ntaygayacn	900
20	gtnacnacna	cngargcnca	ycngcnwsn	carggnaarg	tnaayggna	ngaytggytn	960
	acnytnwsng	cnatgggnat	h				981

primate, e.g., human, OX2RH1 (SEQ ID NO: 14):

25	atgytntgyc	cntggmgna	c ngcnaayytn	ggnytnytny	tnathytnac	nathhtyytn	60
	gtngcngarg	cngarggngc	ngcncarccn	aayaaywsny	tnatgytnca	racnwsnaar	120
	garaaycayg	cnytnngcnws	nwsnwsnytn	tgyatggayg	araarcarat	hacncaraay	180
	taywsnaarg	tnytngcnnga	rgtnaayaacn	wsntggccng	tnaaratggc	nacnaaygcn	240
30	gtnytntgyc	gyccnccnat	hgcnnytnmgn	aayytnatha	thathacntg	ggarathath	300
	ytngmgngnc	arccnwsntg	yacnaargcn	tayaaraarg	aracnaayga	racnaargar	360
	acnaaytgga	cngaygarmg	nathacntgg	gtnwsnmgnc	cngaycaraa	ywsngayytn	420
	carathmgna	cngtngcnat	hacncaygay	ggntaytaym	gntgyathat	ggtnacnccn	480
	gayggnaayt	tycaymgngg	ntaycayytn	cargtnytny	tnacnccnga	rgtnacnytn	540
35	ttycaraaym	gnaaymgna	ngcngtntgy	aargcngtng	cnggnaarcc	ngcngcncay	600
	athwsntgga	thccngargg	ngaytgygcn	acnaarcarg	artaytgows	naayggnaacn	660
	gtnacngtna	arwsnacntg	ycaytgggar	gtncayaayg	tnwsnacngt	nacntgygay	720
	gtnwsncayy	tnacnggnaa	yaarwsnytn	tayathgary	tnytncngt	nccnggngcn	780
	aaraarathw	snaarathat	htaywsnath	taycayccnt	aytaytayta	yytngaycay	840
40	mgnggnathc	ayytngtngt	ngarwsncar	tggytncara	arath		885

primate, e.g., human, OX2RH1.2 (SEQ ID NO: 21):

	atgytntgyc	cntggmgnc	ngcnaaytn	ggnytnytny	tnathytnac	nathttytn	60
	gtngcngarg	cngarggngc	ngcncarccn	aayaaywsny	tnatgytnca	racnwsnaar	120
5	garaaycayg	cnytnngcnws	nwsnwsnytn	tgyatggayg	araarcarat	hacncaraay	180
	taywsnaarg	tnytnngcnga	rgtnaayacn	wsntggccng	tnaaratggc	nacnaaygcn	240
	gtntyntgyt	gyccnccnat	hgcnymngn	aaytnatha	thathacntg	ggarathath	300
	ytmgnggnc	arccnwsntg	yacnaargcn	taymgnaarg	aracnaayga	racnaargar	360
	acnaaytgga	cngaygarmg	nathacntgg	gtnwsnmngc	cngaycaraa	ywsngayytn	420
10	carathmgnc	cngtngcnat	hacncaygay	ggntaytaym	gntgyathat	ggtnacnccn	480
	gayggnaayt	tycaymgngg	ntaycayytn	cargtnytny	tnacnccnga	rgtnacnytn	540
	ttycaraaym	gnaaymgnc	ngcngtntgy	aargcngtng	cnggnaarcc	ngcngcncar	600
	athwsntgga	thccngargg	ngaytgygc	acnaarcarg	artaytggws	naaygggnacn	660
	gtnacngtna	arwsnacntg	ycaytgggar	gtncayaayg	tnwsnacngt	nacntgygay	720
15	gtnwsncayy	tnacnggnaa	yaarwsnytn	tayathgary	tnytnccngt	nccnggngcn	780
	aaraarwsng	cnaarytna	yathccntay	athathytna	cnathathat	hytnacnath	840
	gtnggnttya	thtgyytny	naargtnaay	ggntgymgna	artayaaryt	naayaaracn	900
	garwsnacnc	cngtngtnga	rgargaygar	atgcarcnt	aygcnwsnta	yacngaraar	960
	aayaayccny	tnaygayac	nacnaayaar	gtnaargcnw	sncargcnyt	ncarwsngar	1020
20	gtngayacng	ayytnacayac	nytn				1044

rodent, e.g., mouse, OX2RH1 (SEQ ID NO: 15):

25	atgttytgyt	tytggmgnc	nwsngcnyn	gcngtnytny	tnathtgggg	ngtnttygtn	60
	gcnggnwsnw	sntgyacnga	yaaraaycar	acnacncara	ayaaywsnws	nwsnccnytn	120
	acncargtna	ayacnacngt	nwsngtncar	athgggnacna	argcnytnyt	ntgytgytty	180
	wsnathccny	tnacnaargc	ngtnytnath	acntggatha	thaarytnmg	nggnytnccn	240
	wsntgyacna	thgcntayaa	rgtngayacn	aaracnaayg	aracnwsntg	yytnggngmn	300
30	aayathacnt	ggcnwsnac	nccngaycay	wsnccngary	tnacarathws	ngcngtnacn	360
	ytncarcayg	arggnacnta	yacntgygar	acngtnacnc	cngarggnaa	yttygaraar	420
	aaytaygayy	tncargtnyt	ngtnccnccn	gargtnacnt	ayttyccnga	raaraaymgn	480
	wsngcngtnt	gygargcnat	ggcnggnaar	ccngcngcnc	arathwsntg	gwsnccngay	540
	ggngaytgyg	tnacnacnws	ngarwsncay	wsnaayggna	cngtnacngt	nmgnwsnacn	600
35	tgycaytggg	arcarayaa	ygtnwsngay	gtnwsntgga	thgtnwsnca	yytnacnggn	660
	aaycarwsny	tnwsnathga	rytnwsnmgn	ggnggnaayc	arwsnytnmg	nccntayath	720
	ccntayatha	thccnwsnat	hathathytn	athathathg	gntgyathtg	yytnytnaar	780
	athwsnggnt	tymgnaartg	yaarytnccn	aarytngarg	cnacnwsngc	nathgargar	840
	gaygaratgc	arccntaygc	nwsntayacn	garaarwsna	ayccnytna	ygayacngtn	900
40	acnaargtng	argcnttycc	ngtnwsncar	ggngargtna	ayggnaacnga	ytgyytnacn	960
	ytwnsngcna	thgggnath					978

primate, e.g., human, OX2RH2 (SEQ ID NO: 16):

45	atgggnggna	arcaratgac	ncaraaytay	wsnacnatht	tygcngargg	naayathwsn	60
	carcngtny	tnatggayat	haaygcngtn	ytntgytgyc	cncnathgc	nytnmgnaay	120
	ytathathata	thacntggga	rathathytn	mgnggncarc	cnwsntgyac	naargcntay	180
	aaraargara	cnaaygarac	naargaracn	aaytgyacng	tngarmgnat	hacntgggtn	240
50	wsnmngccng	aycaraayws	ngayytnar	athmgncng	tngayacnac	ncaygayggn	300
	taytaymgng	gnathgtngt	nacnccngay	ggnaayttyc	aymgnggnta	ycayytnar	360
	gtntyngtna	cncngargt	naayytnntt	carwsnmgna	ayathacngc	ngtntgyaar	420
	gcngtnacng	gnaarccngc	ngcncarath	wsntggathc	cngarggnws	nathytnngc	480
	acnaarcarg	artaytgggg	naaygggnacn	gtnacngtna	arwsnacntg	ycntgggar	540
55	ggncayaarw	snacngtnac	ntgycaygtn	wsncayytna	cnggnaayaa	rwsnytnwsn	600
	gtnaarytna	aywsnggnyt	nmgnacnwsn	ggwnsccng	cnytnwsnyt	nytnathath	660
	ytntaygtna	arytnwsnyt	nttygtngtn	athytnngtna	cnacnggntt	ygntnttyty	720
	carmgnatha	aycaygtngm	naargtnytn				750

rodent, e.g., mouse, OX2RH2 (SEQ ID NO: 17):

5	mgnggncarc	cnwsntgyat	hatggcntay	aargtngara	cnaargarac	naaygaracn	60
	tgyytnggnm	gnaayathac	ntgggcnwsn	acncngayc	ayathccnga	yytncarath	120
	wsngcngtng	cnytnarca	ygarggnaay	tayytntgyg	arathacnac	nccngarggn	180
	aayttycaya	argtntayga	yytncargtn	ytngtnccnc	cngargtnac	ntaytttytn	240
	ggngaraaym	gnacngcngt	ntgygargcn	atggcnggna	arccngcngc	ncarathwsn	300
	tggaacncng	ayggngaytg	ygtnacnaar	wsngarwsnc	aywsnaaygg	nacngtnacn	360
10	gtnmgnwsna	cntgycaytg	ggarcaraay	aaygtnwsng	cngtnwsntg	yathgtnwsn	420
	caywsnacng	gnaaycarws	nytnwsnath	garytnwsnm	gnggnacnac	nwsnacnacn	480
	ccnwsnytny	tnacnathyt	ntaygtnaar	atggtnytny	tnggnathat	hytnytnaar	540
	gtnggnttyg	cnttyttyca	raarmgnaay	gtnacnmgna	cn		582

15

rodent, e.g., mouse OX2RH4 (SEQ ID NO: 24):

20	atgcaygcn	tnngnmgnat	hccnacnytn	acnytnytna	thtttyathaa	yathtttygtn	60
	wsnggnwsnw	sntgyacnga	ygaraaycar	acnathcara	aygaywsnws	nwsnwsnytn	120
	acncargtna	ayacnacnat	gwsngtncar	atggayaara	argcnytnyt	ntgytgytty	180
	wsnwsnccny	tnathaaygc	ngtnytnath	acntggatha	thaarcaymg	ncayytncn	240
	wsntgyacna	thgcntayaa	yytngayaar	aaracnaayg	aracnwsntg	yytnggnmgn	300
	aayathacnt	gggcnwsnac	nccngaycay	wsnccngary	tnicarathws	ngcngtngcn	360
	ytncarcayg	arggnacnta	yacntgygar	athgtnacnc	cngarggnaa	yytngaraar	420
25	gtntaygayy	tncargtnyt	ngtnccncn	gargtnacnt	ayttyccngg	naaraaymgn	480
	acngcngtnt	gygargcnat	ggcnggnaar	ccngcngcnc	arathwsntg	gacnccngay	540
	ggngaytggy	tnacnaarws	ngarwsncay	wsnaayggna	cngtnacngt	nmgnwsnacn	600
	tgycaytggy	arcaraayaa	ygtnwsngtn	gtnwsntggy	tngtnwsnca	ywsnacnggn	660
	aaycarwsny	tnwsnathga	rytnwsncar	ggnacnatga	cnacnccnmg	nwsnytnytn	720
30	acnathytnt	aygtnaarat	ggcnytnytn	gtcnathathy	tnytnaaygt	nggnttygcn	780
	tttyttycara	armgnaaytt	ygcnmgnaacn				810

35

rodent, e.g., mouse, OX2RH3 (SEQ ID NO: 18):

40	atgcaygcn	tnngnmgnac	nytnngcnytn	atgytnytna	thtttyathac	nathytnngtn	60
	ccngarwsnw	sntgywsngt	naarggnmgn	gargarathc	cncngayga	ywsnttyccn	120
	ttyswngayg	ayaayathtt	ycngaygggn	gtnggngtna	cnatggarat	hgarathath	180
	acncngtnw	sngtncarat	hggcnathaa	gencarytn	tytgycaycc	nwsnccnwsn	240
	aargargcna	cnytnmgnt	htgggarath	acncnmgng	aytgccnws	ntgymgnytn	300
	ccntaymgng	cngarytnca	rcarathwsn	aaraaratht	gyacngarmg	nggnacnacn	360
	mgngtncng	cncaycayca	rwsnwsngay	ytncnathaa	arwsnatggc	nytnaarcaay	420
	gayggncayt	aywsntgymg	nathgaracn	acngayggna	thttycarga	rmgncaywsn	480
	athcargtnc	cngngarara	ymgnacngtn	gtntgygarg	cnathgcnws	naarccngcn	540
45	atgcarathy	tntggacncc	ngaygargay	tgygtnacna	arwsnaarws	ncayaaygay	600
	acnatgathg	tnmgwnsnaa	rtgycaymgn	garaaraaya	ayggncayws	ngtnttytgy	660
	ttiyathwsnc	ayytnacnga	yaaytggaath	ytwnsnatgg	arcaraaymg	nggnacnacn	720
	wsnathytnc	cnwsnytnyt	nwsnathytn	taygtnaary	tngcngtnac	ngtnytnath	780
50	gtnggnttyg	cnttyttyca	raarmgnaay	taytymgng	tnccngargg	nwsn	834

? = sequence unavailable; "0" = no significant matching

35

The OX2RH1 and 2 embodiments show particular similarity to one another, see, e.g., Table 5. Particular regions or positions of interest are, for the rat H1: boundaries adjacent to (before, at, or after) cys2, leu33, cys35, ile46, trp48, arg53, pro56, cys58, tyr62, cys74, thr80, trp81, leu91, ile93, his100, gly102, tyr104, gly113, phe115, leu122, val123, pro127, asn136, ala139, val140, cys141, ala143, lys147, pro148, ala149, ile152, trp154, pro156, asn169, thr171, val174, ser176, cys178, glu181, ser186, val188, cys190, ser193, his194, thr196, asn198, leu202, gly215, tyr217, leu237, lys238, and ile304. Many of the residues are conserved across the H1 and H2 classes. Likewise with H2 and H4. See Table 5. Particular domains of interest in rat OX2RH1 are the C2 domain from about cys2 to pro127, the C2 domain from about glu128-gly215, the TM segment from about tyr217-leu237, and the intracellular domain from about lys238-ile304. Corresponding segments in mouse H1 are about ser24-pro150, glu151-gly231, tyr239-leu259, and lys260-ile326. In the mouse H2, the segments correspond, in available sequence, from about arg1-pro74, glu75-gly155, pro161-gly182, and phe183-thr194. For human H2, the transmembrane segment is about ala214-val233, and thr234-leu250, and in mouse H3, about pro119-gly237, with the intramembrane lys228, and phe238-gly252. Table 5 also indicates alignment of the H2 and H4 embodiments. Additional positions of interest, e.g., as boundaries for fragments, will be those conserved across homolog groups with the rat OX2RH1 or various subsets of the family members.

Functionally, the rat and mouse H1 have been shown to bind to the OX2. This has not yet been confirmed for the human H1, but can be easily tested. Ligand matching for the H2, H4, and H3 groups is described below.

The rodent H3 has been shown to associate with DAP12, as predicted. Recombinantly expressed epitope tagged DAP12 is not membrane associated in the absence of coexpression of a chaparone partner. see, e.g., Bakker, et al. (1999) Proc. Nat'l Acad. Sci. USA 96:9792-9796. Mouse H3 can serve as the chaparone partner. However, the signal pathway through DAP12 requires binding to the

H3 ligand, which has not yet been identified, but can be found using appropriate screening strategies, e.g., biochemical or physical methods. Sequence similarity of H2 and rodent H4 suggest a similar association with either the DAP12, or possibly the DAP10.

The mouse H2 and H4 and human H2 are likely also to possess such properties, e.g., association with DAP12 (activating) or DAP10. The signaling pathways have been determined with some of the related receptors on NK cells. See, e.g., Lanier, et al. (1998) Immunity 8:693-701; Smith, et al. (1998) J. Immunol. 161:7-10; Gosselin, et al. (1999) J. Leukoc. Biol. 66:165-171; Tomasello, et al. (1998) J. Biol. Chem. 273:34115-34119; and McVicar, et al. (1998) J. Biol. Chem. 273:32934-32942. Because of the similarity of the extracellular domains with the H1 members, OX2-like genes, particularly OX2, are likely ligands.

As used herein, the term OX2RH1, OX2RH2, or OX2RH4 shall be used to describe a protein comprising amino acid sequences shown, e.g., in Tables 1-2. In many cases, a substantial fragment thereof will be functionally or structurally equivalent, including, e.g., an extracellular or intracellular domain. The invention also includes protein variants of the respective OX2RH alleles whose sequences are provided, e.g., muteins or soluble extracellular constructs. Typically, such agonists or antagonists will exhibit less than about 10% sequence differences, and thus will often have between 1 and 11 residue substitutions, e.g., 2, 3, 5, 7, and others. It also encompasses allelic and other variants, e.g., natural polymorphic, of the proteins described. Typically, the receptor will bind to a corresponding biological ligand with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at better than about 3 nM. The term shall also be used herein to refer to related naturally occurring forms, e.g., alleles, polymorphic variants, and metabolic variants of the mammalian proteins. Preferred forms of the receptor complexes will bind the appropriate ligand

with an affinity and selectivity appropriate for a ligand-receptor interaction.

This invention also encompasses combinations of proteins or peptides having substantial amino acid sequence identity with the amino acid sequences in Tables 1-3. It will include sequence variants with relatively few substitutions, e.g., preferably less than about 3-5.

A substantial polypeptide "fragment", or "segment", is a stretch of amino acid residues of at least about 8 amino acids, generally at least 10 amino acids, more generally at least 12 amino acids, often at least 14 amino acids, more often at least 16 amino acids, typically at least 18 amino acids, more typically at least 20 amino acids, usually at least 22 amino acids, more usually at least 24 amino acids, preferably at least 26 amino acids, more preferably at least 28 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids. Sequences of segments of different proteins can be compared to one another over appropriate length stretches. In many situations, fragments may exhibit functional properties of the intact subunits, e.g., the extracellular domain of the transmembrane receptor may retain the ligand binding features, and may be used to prepare a soluble receptor-like complex.

Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches. In some comparisons, gaps may be introduced, as required. See, e.g., Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al. (1983) chapter one in Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison, Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer Group (GCG), Madison, WI; each of which is incorporated herein by reference. This changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine;

and phenylalanine, tyrosine. Homologous amino acid sequences are intended to include natural allelic and interspecies variations in the receptor homolog sequence. Typical homologous proteins or peptides will have from 50-100% homology (if gaps can be introduced), to 60-100% homology (if conservative substitutions are included) with an amino acid sequence segment of Tables 1-3. Homology measures will be at least about 70%, generally at least 76%, more generally at least 81%, often at least 85%, more often at least 88%, typically at least 90%, more typically at least 92%, usually at least 94%, more usually at least 95%, preferably at least 96%, and more preferably at least 97%, and in particularly preferred embodiments, at least 98% or more. The degree of homology will vary with the length of the compared segments. Homologous proteins or peptides, such as the allelic variants, will share most biological activities with the embodiments described in Tables 1-3.

As used herein, the term "biological activity" is used to describe, without limitation, effects on inflammatory responses, innate immunity, and/or morphogenic development by receptor-like proteins. For example, these receptors are likely to mediate their effects through phosphatase or phosphorylase activities, which activities are easily measured by standard procedures. See, e.g., Hardie, et al. (eds. 1995) The Protein Kinase FactBook vols. I and II, Academic Press, San Diego, CA; Hanks, et al. (1991) Meth. Enzymol. 200:38-62; Hunter, et al. (1992) Cell 70:375-388; Lewin (1990) Cell 61:743-752; Pines, et al. (1991) Cold Spring Harbor Symp. Quant. Biol. 56:449-463; and Parker, et al. (1993) Nature 363:736-738. The receptor homologs, or portions thereof, may be useful as phosphate labeling enzymes to label general or specific substrates. The subunits may also be functional immunogens to elicit recognizing antibodies, or antigens capable of binding antibodies.

The terms ligand, agonist, antagonist, and analog of, e.g., an OX2RH, include molecules that modulate the characteristic cellular responses to binding of OX2 proteins, as well as molecules possessing the more standard structural binding

nucleic acid is capable of hybridizing, under appropriate conditions, with a nucleic acid sequence segment shown in Tables 1-3, but preferably not with a corresponding segment of other known Ig superfamily receptors. Said biologically active protein or polypeptide can be a full length protein, or fragment, and will typically have a segment of amino acid sequence highly homologous, e.g., exhibiting significant stretches of identity, to one shown in Tables 1-3. Further, this invention covers the use of isolated or recombinant nucleic acid, or fragments thereof, which encode proteins having fragments which are equivalent to the OX2RH proteins. The isolated nucleic acids can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others from the natural gene.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially pure, e.g., separated from other components which naturally accompany a native sequence, such as ribosomes, polymerases, and flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates, which are thereby distinguishable from naturally occurring compositions, and chemically synthesized analogs or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule, either completely or substantially pure.

An isolated nucleic acid will generally be a homogeneous composition of molecules, but will, in some embodiments, contain heterogeneity, preferably minor. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

A "recombinant" nucleic acid is typically defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence.

Typically this intervention involves in vitro manipulation, although under certain circumstances it may involve more classical animal breeding techniques. Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants as found in their natural state. Thus, for example, products made by transforming cells with an unnaturally occurring vector is encompassed, as are nucleic acids comprising sequence derived using most any synthetic oligonucleotide process. Such a process is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a restriction enzyme sequence recognition site. Alternatively, the process is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms, e.g., encoding a fusion protein. Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, control sequences, or other useful features may be incorporated by design. A similar concept is intended for a recombinant, e.g., fusion, polypeptide. This will include a dimeric repeat. Specifically included are synthetic nucleic acids which, by genetic code redundancy, encode equivalent polypeptides to fragments of OX2 receptor homologs and fusions of sequences from various different related molecules, e.g., other Ig superfamily members.

A "fragment" in a nucleic acid context is a contiguous segment of at least about 17 nucleotides, generally at least 21 nucleotides, more generally at least 25 nucleotides, ordinarily at least 30 nucleotides, more ordinarily at least 35 nucleotides, often at least 39 nucleotides, more often at least 45 nucleotides, typically at least 50 nucleotides, more typically at least 55 nucleotides, usually at least 60 nucleotides, more

usually at least 66 nucleotides, preferably at least 72 nucleotides, more preferably at least 79 nucleotides, and in particularly preferred embodiments will be at least 85 or more nucleotides. Typically, fragments of different genetic sequences can be compared to one another over appropriate length stretches, particularly defined segments such as the domains described below.

A nucleic acid which codes for OX2R homologs will be particularly useful to identify genes, mRNA, and cDNA species which code for itself or closely related proteins, as well as DNAs which code for polymorphic, allelic, or other genetic variants, e.g., from different individuals or related species. Preferred probes for such screens are those regions of the receptor homolog which are conserved between different polymorphic variants or which contain nucleotides which lack specificity, and will preferably be full length or nearly so. In other situations, polymorphic variant specific sequences will be more useful. Quantitation or specific sequence analysis may be useful as markers for disease or medical conditions, or in selecting particular therapeutic treatments.

This invention further covers recombinant nucleic acid molecules and fragments having a nucleic acid sequence identical to or highly homologous to the isolated DNA set forth herein. In particular, the sequences will often be operably linked to DNA segments which control transcription, translation, and/or DNA replication. These additional segments typically assist in expression of the desired nucleic acid segment.

Homologous, or highly identical, nucleic acid sequences, when compared to one another, e.g., OX2RH sequences, exhibit significant similarity. The standards for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison or based upon hybridization conditions. Comparative hybridization conditions are described in greater detail below.

Substantial identity in the nucleic acid sequence comparison context means either that the segments, or their complementary

strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 60% of the nucleotides, generally at least 66%, ordinarily at least 71%, often at least 76%, more often at least 80%, usually at least 84%, more usually at least 88%, typically at least 91%, more typically at least about 93%, preferably at least about 95%, more preferably at least about 96 to 98% or more, and in particular embodiments, as high at about 99% or more of the nucleotides, including, e.g., segments encoding structural domains such as the segments described below. Alternatively, substantial identity will exist when the segments will hybridize under selective hybridization conditions, to a strand or its complement, typically using a sequence derived from Tables 1-3. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, more typically at least about 65%, preferably at least about 75%, and more preferably at least about 90%. See, Kanehisa (1984) Nucl. Acids Res. 12:203-213, which is incorporated herein by reference. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, generally at least about 20 nucleotides, ordinarily at least about 24 nucleotides, usually at least about 28 nucleotides, typically at least about 32 nucleotides, more typically at least about 40 nucleotides, preferably at least about 50 nucleotides, and more preferably at least about 75 to 100 or more nucleotides. This includes, e.g., 125, 150, 175, 200, 225, 246, 273, 300, 325, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, and other lengths.

Stringent conditions, in referring to homology in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters typically controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of about 30° C, more usually in excess of about 37° C, typically in excess of about 45° C, more typically in excess of

about 50° C, e.g., 55° C or 60° C, preferably in excess of about 65° C, and more preferably in excess of about 70° C. Stringent salt conditions will ordinarily be less than about 1 M, more ordinarily less than about 500 mM, usually less than about 400 mM, more usually less than about 300 mM, typically less than about 200 mM, preferably less than about 100 mM, and more preferably less than about 80 mM, e.g., less than 50 mM, even down to less than about 20 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370, which is hereby incorporated herein by reference.

The isolated DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. These modifications generally result in novel DNA sequences which encode this protein or its derivatives. These modified sequences can be used to produce mutant proteins (muteins) or to enhance the expression of variant species. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. Such mutant OX2R homolog derivatives include predetermined or site-specific mutations of the protein or its fragments, including silent mutations using genetic code degeneracy. "Mutant OX2 receptor homolog" as used herein encompasses a polypeptide otherwise falling within the definition of the OX2 receptor homologs as set forth above, but having an amino acid sequence which differs from that of other Ig superfamily as found in nature, whether by way of deletion, substitution, or insertion. In particular, "site specific mutant OX2 receptor homolog" encompasses a protein having substantial sequence identity with a protein of Tables 1-3, and typically shares some or most of the biological activities or effects, e.g., immunogenicity, of the forms disclosed herein.

Although site specific mutation sites are predetermined, mutants need not be site specific. Mammalian OX2 receptor homolog mutagenesis can be achieved by making amino acid insertions or deletions in the gene, coupled with expression.

Substitutions, deletions, insertions, or many combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy- terminal fusions. Random mutagenesis can be conducted at a target codon and the expressed mammalian OX2RH mutants can then be screened for the desired activity, providing some aspect of a structure-activity relationship. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis. See also Sambrook, et al. (1989) and Ausubel, et al. (1987 and periodic Supplements).

The mutations in the DNA normally should not place coding sequences out of reading frames and preferably will not create complementary regions that could hybridize to produce secondary mRNA structure such as loops or hairpins.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Polymerase chain reaction (PCR) techniques can often be applied in mutagenesis. Alternatively, mutagenesis primers are commonly used methods for generating defined mutations at predetermined sites. See, e.g., Innis, et al. (eds. 1990) PCR Protocols: A Guide to Methods and Applications Academic Press, San Diego, CA; and Dieffenbach and Dveksler (1995; eds.) PCR Primer: A Laboratory Manual Cold Spring Harbor Press, CSH, NY.

Certain embodiments of the invention are directed to combination compositions comprising the receptor homolog or ligand sequences described. In other embodiments, functional portions of the sequences may be joined to encode fusion proteins. In other forms, variants of the described sequences may be substituted.

IV. Proteins, Peptides

As described above, the present invention encompasses mammalian OX2RH polypeptides, e.g., whose sequences are disclosed in Tables 1-3, and described above. Allelic and other variant polypeptides are also contemplated, including, e.g., fusion proteins combining portions of such sequences with others, including, e.g., epitope tags, functional domains, and DAP12 or DAP10 sequences.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these primate or rodent proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. Thus, the fusion product of two OX2RHs is a continuous protein molecule having sequences fused in a typical peptide linkage, typically made as a single translation product and exhibiting properties, e.g., sequence or antigenicity, derived from each source peptide. A similar concept applies to heterologous nucleic acid sequences. Combinations of various designated proteins into complexes are also provided.

In addition, new constructs may be made from combining similar functional or structural domains from other related proteins, e.g., other ITIM, ITAM, or YxxM motif containing receptors, including species variants. For example, ligand-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham, et al. (1989) Science 243:1330-1336; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992, each of which is incorporated herein by reference. Thus, new chimeric polypeptides exhibiting new combinations of specificities will result from the functional linkage of receptor-binding specificities. For example, the ligand binding domains from other related receptor homolog molecules may be added or substituted for other domains of this or related proteins. The resulting protein will often have hybrid function and properties. For example, a fusion protein may include a targeting domain which may serve to provide

sequestering of the fusion protein to a particular subcellular organelle.

Candidate fusion partners and sequences can be selected from various sequence data bases, e.g., GenBank, c/o IntelliGenetics, Mountain View, CA; and BCG, University of Wisconsin Biotechnology Computing Group, Madison, WI, which are each incorporated herein by reference. In particular, combinations of polypeptide sequences provided in Tables 1-3 are particularly preferred. Variant forms of the proteins may be substituted in the described combinations.

The present invention particularly provides muteins which bind OX2-like ligands, and/or which are affected in signal transduction. Structural alignment of various members of the OX2 receptor homolog family show conserved features/residues. See, e.g., Table 5. Alignment of the OX2R homolog sequences indicates various structural and functionally shared features. See also, Bazan, et al. (1996) Nature 379:591; Lodi, et al. (1994) Science 263:1762-1766; Sayle and Milner-White (1995) TIBS 20:374-376; and Gronenberg, et al. (1991) Protein Engineering 4:263-269.

Substitutions with either mouse sequences or human sequences are particularly preferred. Conversely, conservative substitutions away from the ligand binding interaction regions will probably preserve most signaling activities; and conservative substitutions away from the intracellular domains will probably preserve most ligand binding properties.

"Derivatives" of a mammalian OX2RH include amino acid sequence mutants, glycosylation variants, metabolic derivatives and covalent or aggregative conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are found in the OX2RH amino acid side chains or at the N- or C- termini, e.g., by means which are well known in the art. These derivatives can include, without limitation, aliphatic esters or amides of the carboxyl terminus, or of residues containing carboxyl side chains, O-acyl derivatives of hydroxyl group-containing residues, and N-acyl derivatives of the amino terminal amino acid or amino-group

containing residues, e.g., lysine or arginine. Acyl groups are selected from the group of alkyl-moieties, including C3 to C18 normal alkyl, thereby forming alkanoyl aroyl species.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. Particularly preferred means for accomplishing this are by exposing the polypeptide to glycosylating enzymes derived from cells which normally provide such processing, e.g., mammalian glycosylation enzymes. Deglycosylation enzymes are also contemplated. Also embraced are versions of the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

A major group of derivatives are covalent conjugates of the receptor homologs or fragments thereof with other proteins of polypeptides. These derivatives can be synthesized in recombinant culture such as N- or C-terminal fusions or by the use of agents known in the art for their usefulness in cross-linking proteins through reactive side groups. Preferred derivatization sites with cross-linking agents are at free amino groups, carbohydrate moieties, and cysteine residues.

Fusion polypeptides between the receptor homologs and other homologous or heterologous proteins are also provided.

Homologous polypeptides may be fusions between different receptors, resulting in, for instance, a hybrid protein exhibiting binding specificity for multiple different OX2 related ligands, or a receptor which may have broadened or weakened specificity of substrate effect. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of a receptor, e.g., a ligand-binding segment, so that the presence or location of a desired ligand may be easily determined. See, e.g., Dull, et al., U.S. Patent No. 4,859,609, which is hereby incorporated herein by reference.

Other gene fusion partners include glutathione-S-transferase (GST), bacterial β -galactosidase, trpE, Protein A, β -lactamase, alpha amylase, alcohol dehydrogenase, and yeast alpha mating factor. See, e.g., Godowski, et al. (1988) Science 241:812-816.

5 Labeled proteins will often be substituted in the described combinations of proteins.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment
10 will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Such polypeptides may also have amino acid residues which
15 have been chemically modified by phosphorylation, sulfonation, biotinylation, or the addition or removal of other moieties, particularly those which have molecular shapes similar to phosphate groups. In some embodiments, the modifications will be useful labeling reagents, or serve as purification targets, e.g.,
20 affinity ligands.

Fusion proteins will typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods. Techniques for nucleic acid manipulation and expression are described generally, for example, in Sambrook, et al. (1989)
25 Molecular Cloning: A Laboratory Manual (2d ed.), Vols. 1-3, Cold Spring Harbor Laboratory, and Ausubel, et al. (eds. 1987 and periodic supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York, which are each incorporated herein by reference. Techniques for synthesis of polypeptides are
30 described, for example, in Merrifield (1963) J. Amer. Chem. Soc. 85:2149-2156; Merrifield (1986) Science 232:341-347; and Atherton, et al. (1989) Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford; each of which is incorporated herein by reference. See also Dawson, et al. (1994)
35 Science 266:776-779 for methods to make larger polypeptides.

This invention also contemplates the use of derivatives of

an OX2RH other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties. These derivatives generally fall into three classes: (1) salts, (2) side chain and terminal residue covalent modifications, and (3) adsorption complexes, e.g., with cell membranes. Such covalent or aggregative derivatives are useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of a receptor or other binding molecule, e.g., an antibody. For example, an OX2 ligand can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated Sepharose, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of an OX2RH, antibodies, or other similar molecules. The ligand can also be labeled with a detectable group, for example radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates, or conjugated to another fluorescent moiety for use in diagnostic assays.

A combination, e.g., including an OX2RH, of this invention can be used as an immunogen for the production of antisera or antibodies specific, e.g., capable of distinguishing between other OX2 receptor homologs, or for desired combination specificity. The OX2RHs can be used to screen monoclonal antibodies or antigen-binding fragments prepared by immunization with various forms of impure preparations containing the protein. In particular, the term "antibodies" also encompasses antigen binding fragments of natural antibodies, e.g., Fab, Fab2, Fv, etc. The purified OX2RH can also be used as a reagent to detect antibodies generated in response to the presence of elevated levels of expression, or immunological disorders which lead to antibody production to the endogenous receptor homolog. Additionally, OX2RH fragments may also serve as immunogens to produce the antibodies of the present invention, as described immediately below. For example, this invention contemplates antibodies having binding affinity to or being raised against the

amino acid sequences shown in Tables 1-3, fragments thereof, or various homologous peptides or subsets. In particular, this invention contemplates antibodies having binding affinity to, or having been raised against, specific fragments which are
 5 predicted to be, or actually are, exposed at the exterior protein surface of the native OX2RH. Complexes of combinations of proteins will also be useful, and antibody preparations thereto can be made.

The blocking of physiological response to the receptor
 10 ligands may result from the inhibition of binding of the ligand to the receptor, likely through competitive inhibition, or perhaps down-regulation of receptor expression due to antibody binding. Thus, in vitro assays of the present invention will often use antibodies or antigen binding segments of these
 15 antibodies, or fragments attached to solid phase substrates. These assays will also allow for the diagnostic determination of the effects of either ligand binding region mutations and modifications, or other mutations and modifications, e.g., which affect signaling or enzymatic function.

This invention also contemplates the use of competitive drug
 20 screening assays, e.g., where neutralizing antibodies to the receptor complexes or fragments compete with a test compound for binding to a ligand or other antibody. In this manner, the neutralizing antibodies or fragments can be used to detect the
 25 presence of a polypeptide which shares one or more binding sites to a receptor and can also be used to occupy binding sites on a receptor that might otherwise bind a ligand.

V. Making Nucleic Acids and Protein

DNA which encodes the protein or fragments thereof can be
 30 obtained by chemical synthesis, screening cDNA libraries, or by screening genomic libraries prepared from a wide variety of cell lines or tissue samples. Natural sequences can be isolated using standard methods and the sequences provided herein, e.g., in
 35 Tables 1-3. Reverse translation sequences are provided in Table 4. Other species counterparts can be identified by hybridization

techniques, or by various PCR techniques, combined with or by searching in sequence databases, e.g., GenBank. Antibodies may be used in expression cloning efforts on species counterparts.

This DNA can be expressed in a wide variety of host cells
5 for the synthesis of a full-length receptor or fragments which can, e.g., be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified ligand binding or kinase/phosphatase domains; and for structure/function studies. Variants or fragments can be
10 expressed in host cells that are transformed or transfected with appropriate expression vectors. These molecules can be substantially free of protein or cellular contaminants, other than those derived from the recombinant host, and therefore are particularly useful in pharmaceutical compositions when combined
15 with a pharmaceutically acceptable carrier and/or diluent. The protein, or portions thereof, may be expressed as fusions with other proteins. Combinations of the described proteins, or nucleic acids encoding them, are particularly interesting.

Expression vectors are typically self-replicating DNA or RNA
20 constructs containing the desired receptor homolog gene or its fragments, usually operably linked to suitable genetic control elements that are recognized in a suitable host cell. These control elements are capable of effecting expression within a suitable host. The multiple genes may be coordinately expressed,
25 and may be on a polycistronic message. The specific type of control elements necessary to effect expression will depend upon the eventual host cell used. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically
30 include a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, and sequences that terminate transcription and translation. Expression vectors also usually
35 contain an origin of replication that allows the vector to replicate independently of the host cell.

The vectors of this invention include those which contain DNA which encodes a combination of proteins, as described, or a biologically active equivalent polypeptide. The DNA can be under the control of a viral promoter and can encode a selection
5 marker. This invention further contemplates use of such expression vectors which are capable of expressing eukaryotic cDNAs coding for such proteins in a prokaryotic or eukaryotic host, where the vector is compatible with the host and where the eukaryotic cDNAs are inserted into the vector such that growth of
10 the host containing the vector expresses the cDNAs in question. Usually, expression vectors are designed for stable replication in their host cells or for amplification to greatly increase the total number of copies of the desirable gene per cell. It is not always necessary to require that an expression vector replicate
15 in a host cell, e.g., it is possible to effect transient expression of the protein or its fragments in various hosts using vectors that do not contain a replication origin that is recognized by the host cell. It is also possible to use vectors that cause integration of the protein encoding portions into the
20 host DNA by recombination.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. Expression vectors are specialized vectors which
25 contain genetic control elements that effect expression of operably linked genes. Plasmids are the most commonly used form of vector but all other forms of vectors which serve an equivalent function and which are, or become, known in the art are suitable for use herein. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier,
30 N.Y., and Rodriguez, et al. (eds. 1988) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworth, Boston, which are incorporated herein by reference.

Transformed cells are cells, preferably mammalian, that have
35 been transformed or transfected with vectors constructed using recombinant DNA techniques. Transformed host cells usually

express the desired proteins, but for purposes of cloning, amplifying, and manipulating its DNA, do not need to express the subject proteins. This invention further contemplates culturing transformed cells in a nutrient medium, thus permitting the
 5 proteins to accumulate. The proteins can be recovered, either from the culture or, in certain instances, from the culture medium.

For purposes of this invention, nucleic acid sequences are operably linked when they are functionally related to each other.
 10 For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the
 15 transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to
 20 operator sequences that in turn control expression.

Suitable host cells include prokaryotes, lower eukaryotes, and higher eukaryotes. Prokaryotes include both gram negative and gram positive organisms, e.g., *E. coli* and *B. subtilis*. Lower eukaryotes include yeasts, e.g., *S. cerevisiae* and *Pichia*,
 25 and species of the genus *Dictyostelium*. Higher eukaryotes include established tissue culture cell lines from animal cells, both of non-mammalian origin, e.g., insect cells, and birds, and of mammalian origin, e.g., human, primates, and rodents.

Prokaryotic host-vector systems include a wide variety of
 30 vectors for many different species. *E. coli* and its vectors will be described, but equivalent vectors and hosts can generally be substituted. A representative vector for amplifying DNA is pBR322 or many of its derivatives. Vectors that can be used to express the receptor homolog or its fragments include, but are
 35 not limited to, such vectors as those containing the lac promoter (pUC-series); trp promoter (pBR322-trp); lpp promoter (the

pIN-series); lambda-pP or pR promoters (pOTS); or hybrid promoters such as ptac (pDR540). See Brosius, et al. (1988) "Expression Vectors Employing Lambda-, trp-, lac-, and Ipp-derived Promoters", in Vectors: A Survey of Molecular Cloning
 5 Vectors and Their Uses, (eds. Rodriguez and Denhardt),
 Buttersworth, Boston, Chapter 10, pp. 205-236, which is incorporated herein by reference.

Lower eukaryotes, e.g., yeasts and Dictyostelium, may be transformed with OX2RH sequence containing vectors. The most
 10 popular lower eukaryotic host is the baker's yeast, *Saccharomyces cerevisiae*. It will be used to exemplify lower eukaryotes, though many other strains and species are also available. Yeast vectors typically consist of a replication origin (unless of the integrating type), a selection gene, a promoter, DNA encoding the
 15 receptor homolog or its fragments, and sequences for translation termination, polyadenylation, and transcription termination. Suitable expression vectors for yeast include such constitutive promoters as 3-phosphoglycerate kinase and various other glycolytic enzyme gene promoters or such inducible promoters as
 20 the alcohol dehydrogenase 2 promoter or metallothionine promoter. Suitable vectors include derivatives of the following types: self-replicating low copy number (such as the YRp-series), self-replicating high copy number (such as the YEp-series); integrating types (such as the YIp-series), or mini-chromosomes
 25 (such as the YCp-series).

Higher eukaryotic tissue culture cells are normally the preferred host cells for expression of functionally active OX2RH proteins. In principle, many higher eukaryotic tissue culture cell lines are workable, e.g., insect baculovirus expression
 30 systems, whether from an invertebrate or vertebrate source. However, mammalian cells are preferred. Transformation or transfection and propagation of such cells has become a routine procedure. Examples of useful cell lines include HeLa cells, Chinese hamster ovary (CHO) cell lines, baby rat kidney (BRK)
 35 cell lines, insect cell lines, bird cell lines, and monkey (COS) cell lines. Expression vectors for such cell lines usually

include an origin of replication, a promoter, a translation initiation site, RNA splice sites (if genomic DNA is used), a polyadenylation site, and a transcription termination site. These vectors also usually contain a selection gene or

5 amplification gene. Suitable expression vectors may be plasmids, viruses, or retroviruses carrying promoters derived, e.g., from such sources as from adenovirus, SV40, parvoviruses, vaccinia virus, or cytomegalovirus. Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136-1142; pMC1neo PolyA, see Thomas, et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610.

For secreted proteins and some membrane proteins, an open reading frame usually encodes a polypeptide that consists of a mature or secreted product covalently linked at its N-terminus to a signal peptide. The signal peptide is cleaved prior to secretion of the mature, or active, polypeptide. The cleavage site can be predicted with a high degree of accuracy from empirical rules, e.g., von-Heijne (1986) Nucleic Acids Research 14:4683-4690, and Nielsen, et al. (1997) Protein Eng. 10:1-12. And the precise amino acid composition of the signal peptide often does not appear to be critical to its function, e.g., Randall, et al. (1989) Science 243:1156-1159; Kaiser, et al. (1987) Science 235:312-317. The mature proteins of the invention can be readily determined using standard methods.

It will often be desired to express these polypeptides in a system which provides a specific or defined glycosylation pattern. In this case, the usual pattern will be that provided naturally by the expression system. However, the pattern will be modifiable by exposing the polypeptide, e.g., an unglycosylated form, to appropriate glycosylating proteins introduced into a heterologous expression system. For example, the OX2RH gene may be co-transformed with one or more genes encoding mammalian or other glycosylating enzymes. Using this approach, certain mammalian glycosylation patterns will be achievable in prokaryote or other cells. Expression in prokaryote cells will typically

lead to unglycosylated forms of protein.

The source of OX2RH can be a eukaryotic or prokaryotic host expressing recombinant polypeptide, such as is described above. The source can also be a cell line, but other mammalian cell
 5 lines are also contemplated by this invention, with the preferred cell line being from the human species.

Now that the sequences are known, the primate OX2RH, fragments, or derivatives thereof can be prepared by conventional processes for synthesizing peptides. These include processes
 10 such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis, Springer-Verlag, New York; and Bodanszky (1984) The Principles of Peptide Synthesis, Springer-Verlag, New York; all of each which
 15 are incorporated herein by reference. For example, an azide process, an acid chloride process, an acid anhydride process, a mixed anhydride process, an active ester process (for example, p-nitrophenyl ester, N-hydroxysuccinimide ester, or cyanomethyl ester), a carbodiimidazole process, an oxidative-reductive
 20 process, or a dicyclohexylcarbodiimide (DCCD)/additive process can be used. Solid phase and solution phase syntheses are both applicable to the foregoing processes. Similar techniques can be used with partial OX2RH sequences.

The OX2RH proteins, fragments, or derivatives are suitably
 25 prepared in accordance with the above processes as typically employed in peptide synthesis, generally either by a so-called stepwise process which comprises condensing an amino acid to the terminal amino acid, one by one in sequence, or by coupling peptide fragments to the terminal amino acid. Amino groups that
 30 are not being used in the coupling reaction typically must be protected to prevent coupling at an incorrect location.

If a solid phase synthesis is adopted, the C-terminal amino acid is bound to an insoluble carrier or support through its carboxyl group. The insoluble carrier should have a binding
 35 capability to a reactive carboxyl group, e.g., halomethyl resins, such as chloromethyl resin or bromomethyl resin, hydroxymethyl

resins, phenol resins, tert-alkyloxycarbonylhydrazidated resins, and the like.

An amino group-protected amino acid is bound in sequence through condensation of its activated carboxyl group and the reactive amino group of the previously formed peptide or chain, to synthesize the peptide step by step. After synthesizing the complete sequence, the peptide is split off from the insoluble carrier to produce the peptide. This solid-phase approach is generally described by Merrifield, et al. (1963) in J. Am. Chem. Soc. 85:2149-2156, which is incorporated herein by reference.

The prepared protein and fragments thereof can be isolated and purified from the reaction mixture by means of peptide separation, e.g., by extraction, precipitation, electrophoresis, various forms of chromatography, and the like. The receptor homologs of this invention can be obtained in varying degrees of purity depending upon desired uses. Purification can be accomplished using standard protein purification techniques or the antibodies herein described in immunoabsorbant affinity chromatography methods. Typically, affinity chromatography is carried out by first linking the antibodies to a solid support and then contacting the linked antibodies with solubilized lysates of appropriate cells, lysates of other cells expressing the OX2RH, or lysates or supernatants of cells producing the protein as a result of DNA techniques, see below.

Generally, the purified protein will be at least about 40% pure, ordinarily at least about 50% pure, usually at least about 60% pure, typically at least about 70% pure, more typically at least about 80% pure, preferable at least about 90% pure and more preferably at least about 95% pure, and in particular embodiments, 97%-99% or more. Purity will usually be on a weight basis, but can also be on a molar basis. Different assays will be applied as appropriate. Individual proteins may be purified and thereafter combined.

VI. Antibodies

Antibodies can be raised to the various mammalian, e.g.,

primate, OX2RH proteins and fragments thereof, both in naturally occurring native forms and in their denatured forms. Antibodies raised to native OX2RH are more likely to recognize epitopes which are only present in the native conformations. Denatured antigen detection can also be useful in, e.g., Western analysis. Anti-idiotypic antibodies are also contemplated, which would be useful, e.g., diagnostic reagents.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of the protein can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective protein, or screened for agonistic or antagonistic activity. These monoclonal antibodies will usually bind with at least a K_D of about 1 mM, more usually at least about 300 μ M, typically at least about 100 μ M, more typically at least about 30 μ M, preferably at least about 10 μ M, and more preferably at least about 3 μ M or better.

The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be potent antagonists that bind to a receptor homolog and inhibit binding to ligand or inhibit the ability of the receptor homolog to elicit a biological response, e.g., act on its substrate. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides to bind OX2RH producing cells. Further, these antibodies can be conjugated to drugs or other therapeutic agents, either directly or indirectly, e.g., by means of a linker.

The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they might bind to the OX2RH without inhibiting ligand or substrate binding. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying ligand. They may be used as reagents for Western blot analysis, or for immunoprecipitation or

immunopurification of the respective protein. Likewise, nucleic acids and proteins may be immobilized to solid substrates for affinity purification or detection methods. The substrates may be, e.g., solid resin beads, sheets of plastic, or derivatized glass.

Protein fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides, to be used as immunogens. Mammalian OX2RH polypeptides and fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See Microbiology, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York; and Williams, et al. (1967) Methods in Immunology and Immunochemistry, Vol. 1, Academic Press, New York; each of which are incorporated herein by reference. A typical method involves hyperimmunization of an animal with an antigen. The blood of the animal is then collected shortly after the repeated immunizations and serum or gamma globulin is isolated.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. See, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.) Academic Press, New York; and particularly Kohler and Milstein (1975) Nature 256:495-497, each of these references is incorporated herein by reference. Briefly, an immunogen is injected into an animal to induce an immune response. The animal is then sacrificed and cells taken from its spleen, which are fused with myeloma cells to produce a hybridoma. The population of hybridomas is then screened to isolate an individual clone which secretes an antibody which binds to the immunogen.

Other suitable techniques involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to

selection of libraries of antibodies in phage or similar vectors. See, Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281; and Ward, et al. (1989) Nature 341:544-546, each of which is hereby incorporated herein by reference. Chimeric or humanized antibodies may be produced, see Cabilly, U.S. Patent No. 4,816,567; or made in transgenic mice, see Mendez, et al. (1997) Nature Genetics 15:146-156. These references are incorporated herein by reference.

Polypeptides and antibodies will often be labeled. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents teaching the use of such labels include, e.g., U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

The antibodies of this invention can also be used for affinity chromatography in isolating the OX2RH proteins or peptides. Columns can be prepared where the antibodies are linked to a solid support, e.g., particles, such as agarose, Sephadex, or the like, where a cell lysate may be passed through the column, the column washed, followed by increasing concentrations of a mild denaturant, whereby the purified protein will be released. Alternatively, the protein may be used to purify antibody. Appropriate cross absorptions or depletions may be applied.

The antibodies may also be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled with a moiety allowing easy detection of presence of antigen by antibody binding.

Antibodies raised against an OX2RH will also be used to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related

to expression of the protein or cells which express the protein. They also will be useful as agonists or antagonists of the ligand, which may be competitive inhibitors or substitutes for naturally occurring ligands.

5 A receptor homolog protein that specifically binds to or that is specifically immunoreactive with an antibody generated against a defined immunogen, such as an immunogen consisting of the amino acid sequence of SEQ ID NO: 2, is typically determined in an immunoassay. The immunoassay typically uses a polyclonal
10 antiserum which was raised, e.g., to a protein of SEQ ID NO: 2. This antiserum is selected to have low crossreactivity against other Ig superfamily receptor members, e.g., NKG2D, preferably from the same species, and any such crossreactivity is removed by immunoabsorption prior to use in the immunoassay.

15 In order to produce antisera for use in an immunoassay, the protein, e.g., of SEQ ID NO: 2, is isolated as described herein. For example, recombinant protein may be produced in a mammalian cell line. An appropriate host, e.g., an inbred strain of mice such as Balb/c, is immunized with the selected protein, typically
20 using a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, supra). Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used as an immunogen. Polyclonal sera are collected and
25 titrated against the immunogen protein in an immunoassay, e.g., a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against other Ig superfamily receptor members using a competitive binding
30 immunoassay such as the one described in Harlow and Lane, supra, at pages 570-573. Preferably at least two receptor family members are used in this determination. These receptor family members can be produced as recombinant proteins and isolated using standard molecular biology and protein chemistry techniques
35 as described herein.

Immunoassays in the competitive binding format can be used

for the crossreactivity determinations. For example, the protein of SEQ ID NO: 2 can be immobilized to a solid support. Proteins added to the assay compete with the binding of the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to the proteins. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with each of the proteins listed above are selected and pooled. The cross-reacting antibodies are then removed from the pooled antisera by immunoabsorption with the above-listed proteins.

The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein to the immunogen protein (e.g., the OX2RH1 like protein of SEQ ID NO: 2). In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required is less than twice the amount of the protein of the selected protein or proteins that is required, then the second protein is said to specifically bind to an antibody generated to the immunogen.

It is understood that these OX2 receptor homolog proteins are members of a family of homologous proteins that comprise at least 6 so far identified genes. For a particular gene product, such as the OX2RH1, the term refers not only to the amino acid sequences disclosed herein, but also to other proteins that are allelic, non-allelic, or species variants. It is also understood that the terms include nonnatural mutations introduced by deliberate mutation using conventional recombinant technology such as single site mutation, or by excising short sections of DNA encoding the respective proteins, or by substituting new amino acids, or adding new amino acids. Such minor alterations typically will substantially maintain the immunoidentity of the original molecule and/or its biological activity. Thus, these alterations include proteins that are specifically immunoreactive

with a designated naturally occurring OX2RH protein. The biological properties of the altered proteins can be determined by expressing the protein in an appropriate cell line and measuring the appropriate effect, e.g., upon transfected lymphocytes. Particular protein modifications considered minor would include conservative substitution of amino acids with similar chemical properties, as described above for the receptor homolog family as a whole. By aligning a protein optimally with the protein of the receptor homologs and by using the conventional immunoassays described herein to determine immunoidentity, one can determine the protein compositions of the invention.

VII. Kits and quantitation

Both naturally occurring and recombinant forms of the receptor like molecules of this invention are particularly useful in kits and assay methods. For example, these methods would also be applied to screening for binding activity, e.g., ligands for these proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds per year. See, e.g., a BIOMEK automated workstation, Beckman Instruments, Palo Alto, California, and Fodor, et al. (1991) Science 251:767-773, which is incorporated herein by reference. The latter describes means for testing binding by a plurality of defined polymers synthesized on a solid substrate. The development of suitable assays to screen for a ligand or agonist/antagonist homologous proteins can be greatly facilitated by the availability of large amounts of purified, soluble receptors in an active state such as is provided by this invention.

Purified OX2RH can be coated directly onto plates for use in the aforementioned ligand screening techniques. However, non-neutralizing antibodies to these proteins can be used as capture antibodies to immobilize the respective receptor homolog on the solid phase, useful, e.g., in diagnostic uses.

This invention also contemplates use of OX2RH, fragments

thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for detecting the presence of the protein or its ligand. Alternatively, or additionally, antibodies against the molecules may be incorporated into the kits and methods and may be used in quantitating the OX2RH or cells expressing them. Typically the kit will have a compartment containing either an OX2RH peptide or gene segment or a reagent which recognizes one or the other. Typically, recognition reagents, in the case of peptide, would be a receptor homolog or antibody, or in the case of a gene segment, would usually be a hybridization probe.

A preferred kit for determining the concentration of OX2RH in a sample would typically comprise a labeled compound, e.g., ligand or antibody, having known binding affinity for OX2RH, a source of OX2RH (naturally occurring or recombinant) as a positive control, and a means for separating the bound from free labeled compound, for example a solid phase for immobilizing the OX2RH in the test sample. Compartments containing reagents, and instructions, will normally be provided. Appropriate nucleic acid or protein containing kits are also provided.

Antibodies, including antigen binding fragments, specific for mammalian OX2RH or a peptide fragment, or receptor homolog fragments are useful in diagnostic applications to detect the presence of elevated levels of homolog and/or its fragments. Diagnostic assays may be homogeneous (without a separation step between free reagent and antibody-antigen complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA) and the like. For example, unlabeled antibodies can be employed by using a second antibody which is labeled and which recognizes the antibody to a receptor homolog or to a particular fragment thereof. These assays have also been extensively discussed in the literature. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH., and

Coligan (ed. 1991 and periodic supplements) Current Protocols In Immunology Greene/Wiley, New York.

Anti-idiotypic antibodies may have similar use to serve as agonists or antagonists of the receptor homologs. These should
5 be useful as therapeutic reagents under appropriate circumstances.

Frequently, the reagents for diagnostic assays are supplied in kits, so as to optimize the sensitivity of the assay. For the
subject invention, depending upon the nature of the assay, the
10 protocol, and the label, either labeled or unlabeled antibody, or labeled ligand is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions
15 for proper use and disposal of the contents after use. Typically the kit has compartments for each useful reagent, and will contain instructions for proper use and disposal of reagents. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium
20 having appropriate concentrations for performing the assay.

The aforementioned constituents of the diagnostic assays may be used without modification or may be modified in a variety of ways. For example, labeling may be achieved by covalently or non-covalently joining a moiety which directly or indirectly
25 provides a detectable signal. In many of these assays, a test compound, receptor homolog, or antibodies thereto can be labeled either directly or indirectly. Possibilities for direct labeling include label groups: radiolabels such as ^{125}I , enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase,
30 and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Both of the patents are incorporated herein by reference. Possibilities for indirect labeling include biotinylation of one constituent followed by
35 binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound from

the free ligand, or alternatively the bound from the free test compound. The receptor homolog can be immobilized on various matrixes followed by washing. Suitable matrices include plastic such as an ELISA plate, filters, and beads. Methods of

5 immobilizing the receptor homolog to a matrix include, without limitation, direct adhesion to plastic, use of a capture antibody, chemical coupling, and biotin-avidin. The last step in this approach involves the precipitation of antibody/antigen complex by any of several methods including those utilizing,

10 e.g., an organic solvent such as polyethylene glycol or a salt such as ammonium sulfate. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30(9):1457-1461, and the double antibody magnetic

15 particle separation as described in U.S. Pat. No. 4,659,678, each of which is incorporated herein by reference.

The methods for linking protein or fragments to various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques

20 involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins

25 will also find use in these applications.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken from the sequence of a receptor homolog. These sequences can be used as probes for detecting levels of the respective receptor homolog in

30 patients suspected of having an immunological disorder. The preparation of both RNA and DNA nucleotide sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. Normally an oligonucleotide probe should have at

35 least about 14 nucleotides, usually at least about 18 nucleotides, and the polynucleotide probes may be up to several

kilobases. Various labels may be employed, most commonly radionuclides, particularly ^{32}P . However, other techniques may also be employed, such as using biotin modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed which can recognize specific duplexes, including DNA duplexes, RNA duplexes, DNA-RNA hybrid duplexes, or DNA-protein duplexes. The antibodies in turn may be labeled and the assay carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected. The use of probes to the novel anti-sense RNA may be carried out in conventional techniques such as nucleic acid hybridization, plus and minus screening, recombinational probing, hybrid released translation (HRT), and hybrid arrested translation (HART). This also includes amplification techniques such as polymerase chain reaction (PCR).

Diagnostic kits which also test for the qualitative or quantitative presence of other markers are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor Res. 1:89-97.

VIII. Therapeutic Utility

This invention provides reagents with significant therapeutic value. See, e.g., Levitzki (1996) Curr. Opin. Cell Biol. 8:239-244. The receptor homologs (naturally occurring or recombinant), fragments thereof, mutein receptors, and antibodies, along with compounds identified as having binding affinity to the receptor homologs or antibodies, should be useful in the treatment of conditions wherein modulation of function of myeloid lineage cells particularly is desirable. Such abnormality will typically be manifested by immunological

disorders, but also by conditions in which myeloid cell activities impact physiological processes, e.g., CNS maturation or development, etc. Additionally, this invention should provide therapeutic value in various diseases or disorders associated with abnormal expression or abnormal triggering of response to the ligand.

In cases where leukocytes, including macrophage/myeloid lineage cells, expressing the OX2R are involved in pathologies and contribute to the disease process, it may be desirable to inhibit the function of these cells. This may be achieved by appropriate stimulation of an OX2R, such that the cell-inhibitory activities of receptor signalling are mobilized. This may be achieved using, e.g., a ligand OX2 agonist or an antibody to the OX2R that has agonistic activities for the receptor. Suitable conditions would be where the animal exhibits signs or symptoms of an inflammatory, leukoproliferative, neurodegenerative, or post-traumatic condition. Preferred embodiments include where the sign or symptom is in neural tissue; lymphoid tissue; myeloid tissue; pancreas; gastrointestinal tissue; thyroid tissue; muscle tissue; or skin or collagenous tissue. Certain embodiments include where the animal is experiencing signs or symptoms of autoimmunity; an inflammatory condition; tissue specific autoimmunity; degenerative autoimmunity; rheumatoid arthritis; atherosclerosis; multiple sclerosis; vasculitides; delayed hypersensitivities; skin grafting; a transplant; spinal injury; stroke; neurodegeneration; or ischemia. The administering agent may be in combination with: an anti-inflammatory cytokine agonist or antagonist; an analgesic; an anti-inflammatory agent; or a steroid.

By contrast, in cases where leukocytes, including macrophage/myeloid lineage cells, expressing the OX2R are involved in processes of immunization and vaccination, repair mechanisms, limiting pathologies, or controlling infection, particularly of bacterial infections, it may be desirable to enhance the function of these cells. This may be achieved therapeutically by appropriate stimulation of an OX2R, such that

the cell-activation activities of receptor signalling are mobilized, or by blocking OX2-OX2R interactions completely should this enable cell-activation to proceed. The latter occurs in ligand OX2 gene knockout mice where the lack of ligand OX2 leads to myeloid cell activation. This may be achieved using, e.g., a ligand OX2 antagonist (such as an antibody against ligand OX2), an antibody to the OX2R that prevents OX2-OX2R interactions, antisense nucleic acids which may prevent OX2R expression, an Ig-OX2R fusion protein that, e.g., by competitive binding, blocks the capacity of cell-bound OX2 to interact with cell-bound OX2R, or a small molecule antagonist. That this modality has applications in vivo in promotion of myeloid cell functions has been demonstrated by experiment, e.g., where i.v. injection to mice of an adenovirus construct producing a human IgG-mouse OX2RH1 fusion protein known to bind mouse OX2 resulted in accelerated onset of the autoimmune disease experimental autoimmune encephalomyelitis (EAE) in these mice, as compared to mice receiving an adenovirus construct producing only the backbone human IgG-fusion protein. The degree of disease acceleration was comparable to that seen in mice in which a ligand for OX2R, namely OX2, had been inactivated by gene targeting.

Alternatively, if the various OX2R molecules described herein have activating vs. inhibitory function, specific activation of the OX2R that induces cellular activation may be appropriate. This may be achieved, e.g., by the use of specific antibody with agonistic activities of a given OX2R. In various embodiments, the method is applied where the animal experiences signs or symptoms of wound healing or clot formation in which enhanced macrophage activation may be desirable, or where an animal experiences a bacterial infection where enhanced phagocytic activity by granulocytes and/or macrophages is desirable. The administering will often be in combination with: an angiogenic factor; a growth factor, including FGF or PDGF; an antibiotic; or a clotting factor.

Recombinant receptors, muteins, agonist or antagonist antibodies thereto, or antibodies can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active ingredients, e.g., in conventional

5 pharmaceutically acceptable carriers or diluents, along with physiologically innocuous stabilizers and excipients. These combinations can be sterile, e.g., filtered, and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also
10 contemplates use of antibodies or binding fragments thereof which are not complement binding.

Ligand screening using receptor or fragments thereof can be performed to identify molecules having binding affinity to the receptors. Subsequent biological assays can then be utilized to
15 determine if a putative ligand can provide competitive binding, which can block intrinsic stimulating activity. Receptor fragments can be used as a blocker or antagonist in that it blocks the activity of ligand. Likewise, a compound having intrinsic stimulating activity can activate the receptor and is
20 thus an agonist in that it simulates the activity of ligand, e.g., inducing signaling. This invention further contemplates the therapeutic use of antibodies to receptors as antagonists.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of
25 administration, target site, reagent physiological life, pharmacological life, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful
30 for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds. 1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics,
35 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; each of

which is hereby incorporated herein by reference. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. Dosage ranges would ordinarily be expected to be in amounts lower than 100 mM concentrations, typically less than about 1 mM concentrations, usually less than about 100 μ M, preferably less than about 1 μ M, and most preferably less than about 10 nM, with an appropriate carrier. Slow release formulations, or slow release apparatus will often be utilized for continuous administration.

Receptor homologs, fragments thereof, and antibodies or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in many conventional dosage formulations. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier must be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds. 1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; Avis, et al. (eds. 1993) Pharmaceutical Dosage

Forms: Parenteral Medications Dekker, NY; Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Tablets Dekker, NY; and Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY. The therapy of this invention may be combined with or used in association with other therapeutic agents, particularly agonists or antagonists of other receptor family members.

IX. Screening

Drug screening using OX2RH or fragments thereof can be performed to identify compounds having binding affinity to the receptor homolog, including isolation of associated components. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of the ligand. Likewise, a compound having intrinsic stimulating activity can activate the receptor and is thus an agonist in that it simulates the activity of a ligand, e.g., OX2. This invention further contemplates the therapeutic use of antibodies to the receptor as agonists or antagonists.

Similarly, complexes comprising multiple proteins may be used to screen for ligands or reagents capable of recognizing the complex. Some receptors comprise at least two subunits, which may be the same, or distinct. Alternatively, the transmembrane receptor may bind to a complex comprising a ligand associated with another soluble protein serving, e.g., as a second receptor subunit.

One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing the OX2RH in combination with another receptor subunit. Cells may be isolated which express a receptor in isolation from other functional receptors. Such cells, either in viable or fixed form, can be used for standard antibody/antigen or ligand/receptor binding assays. See also, Parce, et al. (1989) Science 246:243-247; and Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011, which

describe sensitive methods to detect cellular responses. Competitive assays are particularly useful, where the cells (source of putative ligand) are contacted and incubated with a labeled receptor or antibody having known binding affinity to the ligand, such as ^{125}I -antibody, and a test sample whose binding affinity to the binding composition is being measured. The bound and free labeled binding compositions are then separated to assess the degree of ligand binding. The amount of test compound bound is inversely proportional to the amount of labeled receptor binding to the known source. Many techniques can be used to separate bound from free ligand to assess the degree of ligand binding. This separation step could typically involve a procedure such as adhesion to filters followed by washing, adhesion to plastic followed by washing, or centrifugation of the cell membranes. Viable cells could also be used to screen for the effects of drugs on OX2 mediated functions, e.g., second messenger levels, i.e., Ca^{++} ; cell proliferation; inositol phosphate pool changes; and others. Some detection methods allow for elimination of a separation step, e.g., a proximity sensitive detection system. Calcium sensitive dyes will be useful for detecting Ca^{++} levels, with a fluorimeter or a fluorescence cell sorting apparatus.

X. Ligands

The descriptions of the OX2RHs herein provides means to identify ligands, as described above. Such ligand should bind specifically to the respective receptor with reasonably high affinity. Various constructs are made available which allow either labeling of the receptor to detect its ligand. For example, directly labeling receptor, fusing onto it markers for secondary labeling, e.g., FLAG or other epitope tags, etc., will allow detection of receptor. This can be histological, as an affinity method for biochemical purification, or labeling or selection in an expression cloning approach. A two-hybrid selection system may also be applied making appropriate constructs with the available receptor sequences. See, e.g.,

Fields and Song (1989) Nature 340:245-246.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

EXAMPLES

I. General Methods

5 Some of the standard methods are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols. 1-3, CSH Press, NY; or Ausubel, et al. (1987 and

10 Supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic

15 supplements); Coligan, et al. (ed. 1996) and periodic supplements, Current Protocols In Protein Science Greene/Wiley, New York; Deutscher (1990) "Guide to Protein Purification" in Methods in Enzymology, vol. 182, and other volumes in this

20 series; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow

fusion to appropriate segments, e.g., to a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1990) "Purification of Recombinant Proteins

25 with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QUIAGEN, Inc., Chatsworth, CA.

Computer sequence analysis is performed, e.g., using

30 available software programs, including those from the GCG (U. Wisconsin) and GenBank sources. Public sequence databases were also used, e.g., from GenBank and others.

Many techniques applicable to IL-10 receptors may be applied to the OX2RHs, as described, e.g., in USSN 08/110,683 (IL-10

35 receptor), which is incorporated herein by reference.

II. Monoclonal Antibody which blocks rat OX2/OX2RH interaction on macrophages

A bead assay was set up using recombinant OX2-CD4 protein and rat peritoneal macrophages. See Preston, et al. (1997) Eur. J. Immunol. 27:1911-1918. Macrophages bound to fluorescent beads coated with recombinant OX2-CD4 proteins. Six wk old BALB/c mice were immunized 6 times with either 0.1-0.25 mg crude membrane fraction (Williams and Barclay (1986) in Handbook of Experimental Immunology vol. 1, 22.1-22.24, Blackwell Scientific Publications) or resident rat peritoneal exudate cells (5 million). Mice were screened for high titers of antibodies recognizing macrophages by testing various dilutions of the sera for labeling of macrophages by indirect immunofluorescence and flow cytometry. Mice producing good immune responses to the rat macrophages were finally boosted by injection of peritoneal exudate cells. Four days later, spleens were removed and fused to NS-1 myeloma cells to produce hybridomas. The final injection before screening was intrasplenic. Hybridoma supernatants were screened for the ability to label rat macrophages and for the ability to block the rat OX2 interaction with macrophages. One antibody, designated OX102, was obtained and cloned. This antibody gave clear blocking. This hybridoma was grown in bulk and the antibody was purified by standard procedures.

III. Purification of the antigen for the OX102 mAb

Purified OX102 mAb was covalently coupled to CNBr activated sepharose-4B (Pharmacia) as recommended by the manufacturer. Membrane proteins were solubilised using Tween 40 and sodium deoxycholate and incubated with the Sepharose beads coupled to OX102 mAb, for 70 hours. Williams and Barclay (1986) in Handbook of Experimental Immunology vol. 1, 22.1-22.24, Blackwell Scientific Publications. The OX102 mAb-coupled Sepharose beads were pelleted by centrifugation and washed in 0.1% sodium dodecyl sulphate (SDS) and finally eluted in 0.5% SDS at 55° C for 15 min. The eluted fraction was analysed by SDS polyacrylamide gel electrophoresis (SDS-PAGE).

IV. N-terminal sequence of the antigen for the OX102 mAb

Amino terminal sequencing was performed using automated Edman degradation in an Applied Biosystems Procise 494A protein sequencer (Perkin-Elmer Ltd., UK). The N-terminal sequence was confirmed, as shown in Table 1. Blank cycles are assumed to be asparagine due to the presence of asparagine modified by N-linked glycosylation. The purified polypeptide was identified as novel by screening known protein databases with the N-terminal 20 amino acids of the antigen for the OX102 mAb. This protein is the rat OX2RH1.

V. Isolation of cDNA clones coding for the antigen of the OX102 mAb

Total RNA was extracted from rat peritoneal exudate cells using RNazol B (Biogenesis) and then the poly-A fraction purified using oligo dT beads (Oligotex, QIAGEN) as recommended by the manufacturer. Approximately 50 ng of polyA+ purified mRNA was treated with 200 U of Superscript II reverse transcriptase (GIBCO BRL) in the presence of 1 μ M of selected sense and antisense oligonucleotides, 1 mM dNTPs, and 2 mM DTT, 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl₂, and incubated at 42° C for 1 h.

This cDNA was then used as a template in a PCR reaction, e.g., 40 μ l of provided 10x Advantage Taq thermophilic PCR buffer (provided by Clontech); 8 μ l of 10 mM dNTPs; 8 μ l of Advantage Taq (Clontech); 2 μ l of cDNA prepared as described above; 318 μ l of distilled water; 16 μ l of an antisense, degenerate oligonucleotide corresponding to the N-terminal peptide at 10 μ M; and 8 μ l 10 μ M sense oligonucleotide. Both oligonucleotide primers were synthesized (Genosys) with a 5' terminal phosphate to facilitate cloning.

The PCR mix was aliquoted into 8 x 50 μ l samples and subjected to PCR conditions in a Robocycler PCR machine (Stratagene) which allows the operator to vary the annealing temperature in separate samples simultaneously. Example parameters are: 93° C 30 sec; followed by 35 cycles of: 93° C 30

sec; 42–56° C 1 min; 72° C 30 sec; and a final cycle of 72° C for 8 min.

Ten µl of the PCR products were analyzed by agarose gel electrophoresis by standard procedures. PCR products of lengths ranging between 100 and 300 base pairs in the 3 samples which had an annealing temperature of about 42°, 44°, and 46° C were excised from the gel and the nucleic acid purified using QIAquick (QIAGEN). These purified products were ligated at about 16° C for 48 h using standard procedures into PCRScript vector (Stratagene) which had been SmaI digested and phosphatase treated.

Transformants were screened initially by colony PCR and 20 colonies containing appropriately-sized inserts were grown up in LB broth in the presence of 50 µg/ml ampicillin and plasmids purified by a QIAGEN robot. Inserts were sequenced using the BIGDYE fluorescent dideoxy-terminator technology and ABI-PRISM Model 377 (Perkin-Elmer Ltd., UK). Inserts containing nucleotide sequence that coded for the N-terminal sequence of antigen for the OX102 mAb were used to design oligonucleotides for 3' RACE reactions.

The full cDNA sequence of the antigen for OX102 was obtained by 3'RACE PCR (using the same protocol as above) but modified by using appropriate oligonucleotides at a final concentration of 0.2 µM each. PCR conditions, e.g., were: 93° C 30 sec; followed by 30 cycles of: 93° C 30 sec; 51°–65° C 1 min; 72° C 3.5 min; and a final cycle of 72° C for 12 min.

A band of approximately 2.3 Kb was excised from the 65° C PCR reaction, gel purified, digested with NotI and XhoI, and ligated into NotI/XhoI digested vector (PCRScript, Stratagene) using standard procedures. Inserts were sequenced as above.

The cDNA sequence of the OX102 protein, also referred to herein as rat OX2RH1, is shown in Table 1. Full length isolates of the other homolog embodiments are similarly cloned and sequences confirmed. Standard methods are readily applicable.

VI. Obtaining other OX2RH cDNAs

The knowledge of the rat OX2RH1 nucleotide and predicted amino acid sequences allows one to obtain homologous functional equivalents from other species, including mouse or human OX2RH1, on the basis of sequence similarity and because the rat OX2RH1 provides a tool for the isolation of such equivalents.

Thus, to identify human OX2RH1, one can search existing databases of nucleotide and amino acid sequences for polypeptides of unknown identity (e.g., databases storing sequences obtained from the Human Genome Project) for sequences with homology to the rat OX2RH1 nucleic acid and amino acid sequences provided herein. The databases, which are stored and updated at many sites, including the European Bioinformatics Centre (<http://www.ebi.ac.uk/>) and National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>), can be accessed by widely-used programs such as FASTA or BLAST. These databases include sequences for expressed sequence tags (ESTs) which are short regions of nucleotide sequence sequenced from random cDNA clones. This allows partial cDNA clones for mouse or human OX2RH to be isolated by comparison with the rat OX2RH sequence information provided herein. Full length clones can then be isolated by screening macrophage cDNA or genomic libraries or by primer extension techniques such as those described herein for obtaining the full-length rat OX2RH1 clones.

Mouse and human sequences related to the rat OX2RH1 were identified from genomic sequence database using, e.g., the BLAST server (Altschul, et al. (1994) Nature Genet. 6:119-129). Standard analysis programs may be used to evaluate structure, e.g., PHD (Rost and Sander (1994) Proteins 19:55-72) and DSC (King and Sternberg (1996) Protein Sci. 5:2298-2310). Standard comparison software includes, e.g., Altschul, et al. (1990) J. Mol. Biol. 215:403-10; Waterman (1995) Introduction to Computational Biology: Maps, Sequences, and Genomes Chapman & Hall; Lander and Waterman (eds. 1995) Calculating the Secrets of Life: Applications of the Mathematical Sciences in Molecular Biology National Academy Press; and Speed and Waterman (eds.

1996) Genetic Mapping and DNA Sequencing (IMA Volumes in Mathematics and Its Applications, Vol 81) Springer Verlag.

Nucleic acid sequence for rat and human OX2RH1 will have between 50 and 98% homology, e.g., as can be observed in Table 5.

5 For the other homologs, the similarity may be less, especially with the homolog 3.

As an alternative to database screening, the rat OX2RH1 nucleic acid sequence as provided herein can be used to screen macrophage cDNA or genomic libraries to identify human sequences sufficiently homologous to hybridize under conditions of appropriate stringency. This approach was utilized to isolate the human OX2 gene using rat OX2 nucleic acid as a probe. McCaughan, et al. (1987) Immunogenetics 25:329-335). PCR based methods using templates of cDNA, genomic DNA, cDNA clones, or genomic clones as templates are also widely used, the general approach being exemplified by the isolation of mouse OX2 from cDNA. See Preston, et al. (1997) Eur. J. Immunol. 27:1911-1918.

PCR primers derived from the provided OX2RH sequences are used to probe a human, or other species or tissue, cDNA library. Sequences may be derived, e.g., from Tables 1-4, preferably those adjacent the ends of sequences. Full length cDNAs for primate, rodent, or other species OX2RHs are cloned, e.g., by DNA hybridization screening of λ gt10 phage. PCR reactions are conducted using T. aquaticus Taqplus DNA polymerase (Stratagene) under appropriate conditions.

Further, the rat OX2RH1 sequence can be used to isolate the corresponding mouse OX2RH1 sequence, and to identify regions conserved between them. Particularly with the discovery of a group of homologs, regions of similarity may be identified. Conserved-region sequences provide useful reagents for identifying a given gene in a range of species. For instance domain 1 of mouse and rat OX2 are 90% identical at the amino acid level, compared to 77% identity between the same human and rat domains. Preston, et al. (1997) Eur. J. Immunol. 27:1911-1918.

35 Yet another method is to use antibody reagents to expression clone or identify crossreacting proteins expressed in cDNA

libraries from appropriate cell types, e.g., macrophages, or from other species.

VII. Chromosomal localization

5 The genes will be mapped. For example, chromosome spreads are prepared. In situ hybridization is performed on chromosome preparations obtained from phytohemagglutinin-stimulated lymphocytes, e.g., from human, cultured for 72 h. 5-bromodeoxyuridine is added for the final seven hours of culture
10 (60 µg/ml of medium), to ensure a posthybridization chromosomal banding of good quality.

A PCR fragment, amplified with the help of primers, is cloned into an appropriate vector. The vector is labeled by nick-translation with ³H. The radiolabeled probe is hybridized
15 to metaphase spreads at final concentration of 200 ng/ml of hybridization solution as described in Mattei, et al. (1985) Hum. Genet. 69:327-331.

After coating with nuclear track emulsion (KODAK NTB2), slides are exposed. To avoid any slipping of silver grains
20 during the banding procedure, chromosome spreads are first stained with buffered Giemsa solution and metaphase photographed. R-banding is then performed by the fluorochrome-photolysis-Giemsa (FPG) method and metaphases rephotographed before analysis.

Similar appropriate methods are used for other species.
25

VIII. Localization of various OX2RH mRNA

While the expected expression patterns of the OX2RH1 are primarily on macrophages, granulocytes, and mast cells, the homologs H2, H3, and/or H4 may not be so closely related
30 functionally. Thus, the distribution of those will be of particular interest. Distribution may be evaluated at the nucleic acid level, e.g., by hybridization or PCR methods, or at the protein level, e.g., by histology or immunochemical methods.

Human multiple tissue (Cat #1, 2) and cancer cell line blots
35 (Cat #7757-1), containing approximately 2 µg of poly(A)⁺ RNA per lane, are purchased from Clontech (Palo Alto, CA). Probes are

radiolabeled with [α - 32 P] dATP, e.g., using the Amersham Rediprime random primer labeling kit (RPN1633). Prehybridization and hybridizations are performed, e.g., at 65° C in 0.5 M Na₂HPO₄, 7% SDS, 0.5 M EDTA (pH 8.0). High stringency washes are
 5 conducted, e.g., at 65° C with two initial washes in 2 x SSC, 0.1% SDS for 40 min followed by a subsequent wash in 0.1 x SSC, 0.1% SDS for 20 min. Membranes are then exposed at -70° C to X-Ray film (Kodak) in the presence of intensifying screens. More detailed studies by cDNA library Southernns are performed with
 10 selected appropriate mammalian OX2RH clones to examine their expression in hemopoietic or other cell subsets.

Alternatively, two appropriate primers are selected, e.g., from Tables 1-4. RT-PCR is used on an appropriate mRNA sample selected for the presence of message to produce a cDNA, e.g., a
 15 sample which expresses the gene.

Full length clones may be isolated by hybridization of cDNA libraries from appropriate tissues pre-selected by PCR signal. Northern blots can be performed.

Message for genes encoding OX2RHs will be assayed by
 20 appropriate technology, e.g., PCR, immunoassay, hybridization, or otherwise. Tissue and organ cDNA preparations are available, e.g., from Clontech, Mountain View, CA. Identification of sources of natural expression are useful, as described.

For mouse distribution, e.g., Southern Analysis can be
 25 performed: DNA (5 μ g) from a primary amplified cDNA library was digested with appropriate restriction enzymes to release the inserts, run on a 1% agarose gel and transferred to a nylon membrane (Schleicher and Schuell, Keene, NH).

Samples for mouse mRNA isolation may include: resting mouse
 30 fibroblastic L cell line (C200); Braf:ER (Braf fusion to estrogen receptor) transfected cells, control (C201); T cells, TH1 polarized (Mell4 bright, CD4+ cells from spleen, polarized for 7 days with IFN- γ and anti IL-4; T200); T cells, TH2 polarized (Mell4 bright, CD4+ cells from spleen, polarized for 7 days with
 35 IL-4 and anti-IFN- γ ; T201); T cells, highly TH1 polarized (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated

with anti-CD3 for 2, 6, 16 h pooled; T202); T cells, highly TH2 polarized (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated with anti-CD3 for 2, 6, 16 h pooled; T203); CD44-CD25+ pre T cells, sorted from thymus (T204); TH1 T cell clone D1.1, resting for 3 weeks after last stimulation with antigen (T205); TH1 T cell clone D1.1, 10 µg/ml ConA stimulated 15 h (T206); TH2 T cell clone CDC35, resting for 3 weeks after last stimulation with antigen (T207); TH2 T cell clone CDC35, 10 µg/ml ConA stimulated 15 h (T208); Mell14+ naive T cells from spleen, resting (T209); Mell14+ T cells, polarized to Th1 with IFN-γ/IL-12/anti-IL-4 for 6, 12, 24 h pooled (T210); Mell14+ T cells, polarized to Th2 with IL-4/anti-IFN-γ for 6, 13, 24 h pooled (T211); unstimulated mature B cell leukemia cell line A20 (B200); unstimulated B cell line CH12 (B201); unstimulated large B cells from spleen (B202); B cells from total spleen, LPS activated (B203); metrizamide enriched dendritic cells from spleen, resting (D200); dendritic cells from bone marrow, resting (D201); monocyte cell line RAW 264.7 activated with LPS 4 h (M200); bone-marrow macrophages derived with GM and M-CSF (M201); macrophage cell line J774, resting (M202); macrophage cell line J774 + LPS + anti-IL-10 at 0.5, 1, 3, 6, 12 h pooled (M203); macrophage cell line J774 + LPS + IL-10 at 0.5, 1, 3, 5, 12 h pooled (M204); aerosol challenged mouse lung tissue, Th2 primers, aerosol OVA challenge 7, 14, 23 h pooled (see Garlisi, et al. (1995) Clinical Immunology and Immunopathology 75:75-83; X206); Nippostrongylus-infected lung tissue (see Coffman, et al. (1989) Science 245:308-310; X200); total adult lung, normal (O200); total lung, rag-1 (see Schwarz, et al. (1993) Immunodeficiency 4:249-252; O205); IL-10 K.O. spleen (see Kuhn, et al. (1991) Cell 75:263-274; X201); total adult spleen, normal (O201); total spleen, rag-1 (O207); IL-10 K.O. Peyer's patches (O202); total Peyer's patches, normal (O210); IL-10 K.O. mesenteric lymph nodes (X203); total mesenteric lymph nodes, normal (O211); IL-10 K.O. colon (X203); total colon, normal (O212); NOD mouse pancreas (see Makino, et al. (1980) Jikken Dobutsu 29:1-13; X205); total thymus, rag-1 (O208); total kidney, rag-1 (O209); total heart, rag-1 (O202);

total brain, rag-1 (O203); total testes, rag-1 (O204); total liver, rag-1 (O206); rat normal joint tissue (O300); and rat arthritic joint tissue (X300).

Samples for human mRNA isolation may include: peripheral
5 blood mononuclear cells (monocytes, T cells, NK cells, granulocytes, B cells), resting (T100); peripheral blood mononuclear cells, activated with anti-CD3 for 2, 6, 12 h pooled (T101); T cell, TH0 clone Mot 72, resting (T102); T cell, TH0 clone Mot 72, activated with anti-CD28 and anti-CD3 for 3, 6, 12
10 h pooled (T103); T cell, TH0 clone Mot 72, anergic treated with specific peptide for 2, 7, 12 h pooled (T104); T cell, TH1 clone HY06, resting (T107); T cell, TH1 clone HY06, activated with anti-CD28 and anti-CD3 for 3, 6, 12 h pooled (T108); T cell, TH1 clone HY06, anergic treated with specific peptide for 2, 6, 12 h
15 pooled (T109); T cell, TH2 clone HY935, resting (T110); T cell, TH2 clone HY935, activated with anti-CD28 and anti-CD3 for 2, 7, 12 h pooled (T111); T cells CD4+CD45RO- T cells polarized 27 days in anti-CD28, IL-4, and anti IFN- γ , TH2 polarized, activated with anti-CD3 and anti-CD28 4 h (T116); T cell tumor lines Jurkat and
20 Hut78, resting (T117); T cell clones, pooled AD130.2, Tc783.12, Tc783.13, Tc783.58, Tc782.69, resting (T118); T cell random $\gamma\delta$ T cell clones, resting (T119); Splenocytes, resting (B100); Splenocytes, activated with anti-CD40 and IL-4 (B101); B cell EBV lines pooled WT49, RSB, JY, CVIR, 721.221, RM3, HSY, resting
25 (B102); B cell line JY, activated with PMA and ionomycin for 1, 6 h pooled (B103); NK 20 clones pooled, resting (K100); NK 20 clones pooled, activated with PMA and ionomycin for 6 h (K101); NK1 clone, derived from peripheral blood of LGL leukemia patient, IL-2 treated (K106); NK cytotoxic clone 640-A30-1, resting
30 (K107); hematopoietic precursor line TF1, activated with PMA and ionomycin for 1, 6 h pooled (C100); U937 premonocytic line, resting (M100); U937 premonocytic line, activated with PMA and ionomycin for 1, 6 h pooled (M101); elutriated monocytes, activated with LPS, IFN γ , anti-IL-10 for 1, 2, 6, 12, 24 h pooled
35 (M102); elutriated monocytes, activated with LPS, IFN γ , IL-10 for 1, 2, 6, 12, 24 h pooled (M103); elutriated monocytes, activated

with LPS, IFN γ , anti-IL-10 for 4, 16 h pooled (M106); elutriated monocytes, activated with LPS, IFN γ , IL-10 for 4, 16 h pooled (M107); elutriated monocytes, activated LPS for 1 h (M108); elutriated monocytes, activated LPS for 6 h (M109); DC 70% CD1a+, from CD34+ GM-CSF, TNF α 12 days, resting (D101); DC 70% CD1a+, from CD34+ GM-CSF, TNF α 12 days, activated with PMA and ionomycin for 1 hr (D102); DC 70% CD1a+, from CD34+ GM-CSF, TNF α 12 days, activated with PMA and ionomycin for 6 hr (D103); DC 95% CD1a+, from CD34+ GM-CSF, TNF α 12 days FACS sorted, activated with PMA and ionomycin for 1, 6 h pooled (D104); DC 95% CD14+, ex CD34+ GM-CSF, TNF α 12 days FACS sorted, activated with PMA and ionomycin 1, 6 hr pooled (D105); DC CD1a+ CD86+, from CD34+ GM-CSF, TNF α 12 days FACS sorted, activated with PMA and ionomycin for 1, 6 h pooled (D106); DC from monocytes GM-CSF, IL-4 5 days, resting (D107); DC from monocytes GM-CSF, IL-4 5 days, resting (D108); DC from monocytes GM-CSF, IL-4 5 days, activated LPS 4, 16 h pooled (D109); DC from monocytes GM-CSF, IL-4 5 days, activated TNF α , monocyte supe for 4, 16 h pooled (D110); leiomyoma L11 benign tumor (X101); normal myometrium M5 (O115); malignant leiomyosarcoma GS1 (X103); lung fibroblast sarcoma line MRC5, activated with PMA and ionomycin for 1, 6 h pooled (C101); kidney epithelial carcinoma cell line CHA, activated with PMA and ionomycin for 1, 6 h pooled (C102); kidney fetal 28 wk male (O100); lung fetal 28 wk male (O101); liver fetal 28 wk male (O102); heart fetal 28 wk male (O103); brain fetal 28 wk male (O104); gallbladder fetal 28 wk male (O106); small intestine fetal 28 wk male (O107); adipose tissue fetal 28 wk male (O108); ovary fetal 25 wk female (O109); uterus fetal 25 wk female (O110); testes fetal 28 wk male (O111); spleen fetal 28 wk male (O112); adult placenta 28 wk (O113); and tonsil inflamed, from 12 year old (X100).

Similar samples may isolated in other species for evaluation. Histology may also be performed.

Comparison of the CD spectrum with similar Ig superfamily receptor protein may suggest that the protein is correctly folded. See Hazuda, et al. (1969) J. Biol. Chem. 264:1689-1693; and Campbell, et al. (1979) Nature 282:341-342.

5 The reactivity of the OX2/OX2R in terms of binding properties, e.g., kinetics and functional effects, can be investigated. The interactions of the OX2R cytoplasmic domain can be determined using well established immunoprecipitation methods or genetic methods such as the yeast two-hybrid system.
10 Transfection of the OX2RH into cells normally not expressing the proteins may be useful in physiological and signaling studies.

X. Preparation of antibodies specific for OX2RHs

Appropriate species or strains, e.g., inbred Balb/c mice,
15 are immunized intraperitoneally with recombinant forms of the protein, e.g., purified OX2RH or stable transfected NIH-3T3 cells. Animals are boosted at appropriate time points with protein, with or without additional adjuvant, to further stimulate antibody production. Serum is collected, or hybridomas
20 produced with harvested spleens.

Alternatively, the animals, e.g., Balb/c mice, are immunized with cells transformed with the gene or fragments thereof, either endogenous or exogenous cells, or with isolated membranes enriched for expression of the antigen. Serum is collected at
25 the appropriate time, typically after numerous further administrations. Various gene therapy techniques may be useful, e.g., in producing protein in situ, for generating an immune response. Serum or antibody preparations may be cross-absorbed or immunoselected to prepare substantially purified antibodies of
30 defined specificity and high affinity. Thus, antibodies could be prepared which recognize various species counterparts, or antibodies which recognize specific species or groups of subsets, e.g., rodent, embodiments.

Monoclonal antibodies may be made. For example, splenocytes
35 are fused with an appropriate fusion partner and hybridomas are selected in growth medium by standard procedures. Hybridoma

supernatants are screened for the presence of antibodies which bind to the rat OX2RH1, e.g., by ELISA or other assay. Antibodies which specifically recognize specific OX2RH embodiments may also be selected or prepared.

- 5 In another method, synthetic peptides or purified protein are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (ed. 1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. In
10 appropriate situations, the binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods. Nucleic acids may also be introduced into cells in an animal to produce the antigen, which serves to elicit an immune response. See, e.g., Wang, et al.
15 (1993) Proc. Nat'l. Acad. Sci. 90:4156-4160; Barry, et al. (1994) BioTechniques 16:616-619; and Xiang, et al. (1995) Immunity 2: 129-135.

XI. Ligand binding and partner specificity

- 20 Means for testing of the binding selectivity and affinity are readily available. Surface plasmon resonance (see manufacturer's protocol; BIAcore manual, Pharmacia Biosensor) or other methods may be used to determine the ligand for the OX2RHs. The rat and mouse H1 bind to their species counterpart ligand
25 OX2; the human H1 will be similarly tested. The H2 will be similarly tested against similar potential ligands, though the similarity of the extracellular domains of the H2 to the known receptor (rat and mouse H1) suggest the same or closely related ligand.
- 30 A receptor can be used as a specific binding reagent to identify its binding partner, by taking advantage of its specificity of binding, much like an antibody would be used. The binding receptor may be an OX2RH, or may involve, e.g., a complex of the OX2RH with another subunit. A binding reagent is either
35 labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods.

The binding composition is used to screen an expression library made from a cell line which expresses a binding partner, i.e., ligand, preferably membrane associated. Standard staining techniques are used to detect or sort surface expressed ligand, or surface expressing transformed cells are screened by panning. Screening of intracellular expression is performed by various staining or immunofluorescence procedures. See also McMahan, et al. (1991) EMBO J. 10:2821-2832.

Alternatively, receptor reagents are used to affinity purify or sort out cells expressing a putative ligand. See, e.g., Sambrook, et al. or Ausubel, et al.

Another strategy is to screen for a membrane bound ligand by panning. The receptor cDNA is constructed as described above. Immobilization may be achieved by use of appropriate antibodies which recognize, e.g., a FLAG sequence on an OX2RH fusion construct, or by use of antibodies raised against the first antibodies. Recursive cycles of selection and amplification lead to enrichment of appropriate clones and eventual isolation of receptor expressing clones.

Phage expression libraries can be screened by mammalian OX2RH, e.g., labeled forms. Appropriate label techniques, e.g., anti-FLAG antibodies, will allow specific labeling of appropriate phage clones.

Upon confirmation of OX2-OX2RH binding, or identification of alternative ligands for the other homologs, signaling pathways will be tested. See, e.g., Preston, et al. (1997) Eur. J. Immunol. 27:1911-1918. Implications of the DAP12 involvement are also clear. See, e.g., Bakker, et al. (2000) Human Immunology 61:18-27; Lanier, et al. (1998) Immunity 8:693-701; Smith, et al. (1998) J. Immunol. 161:7-10; Gosselin, et al. (1999) J. Leukoc. Biol. 66:165-171; Tomasello, et al. (1998) J. Biol. Chem. 273:34115-34119; and McVicar, et al. (1998) J. Biol. Chem. 273:32934-32942. Similarly, or alternatively, DAP10 may be involved. See, e.g., Wu J, et al. (1999) Science 285:730-732; and Bauer, et al. (1999) Science 285:727-729.

In particular, the DAP12 coreceptor partner is in the same family as the T cell receptor subunit ζ and the Fc ϵ R γ , which possess ITIM motifs, and signal through the pathway involving the syk/zap70 protein tyrosine kinases. The DAP10 has the YxxM motif, which signals through or analogously to the PI3 kinase pathway.

Certain isoforms of the MHC class I receptors on NK cells lack ITIM sequences in their cytoplasmic domains and it has been proposed that these isoforms activate, rather than inhibit, NK cells. These activating receptors have very short intracellular regions lacking any signaling motifs and they all share a positively charged residue within their transmembrane domain, which suggested the association with an adapter molecule that is capable of signaling. DAP12, a type I disulfide-linked homodimer containing an ITAM non-covalently assembles with the human KIR2DS receptors. DAP12 has a negatively charged aspartic acid residue in its transmembrane region and corresponds to a reported 12-13 kD phosphoprotein that was found to co-immunoprecipitate with KIR2DS.

Upon receptor engagement, DAP12 becomes phosphorylated and recruits the Syk kinase, thus inducing a signaling cascade similar to T cell receptor. Besides being associated with KIR2DS, a receptor for HLA-C, DAP12 is also expressed at the cell surface of NK cells associated with the activating mouse Ly49D and Ly49H receptors recognizing H-2 and with the human CD94/NKG2C heterodimer receptor complex recognizing HLA-E.

Recent efforts to identify potential membrane signaling proteins by searching the EST databases have led to the identification of DAP10, a novel 10-kD surface adapter primarily expressed in hematopoietic cells. Although DAP10 has only limited homology with DAP12, its transmembrane domain contains a negatively charged residue that is conserved in the transmembrane regions of DAP12 and all of the CD3 subunits of the TCR. In addition, the conserved cysteine residues within the extracellular domain of DAP12 and the CD3 chains are also present in DAP10. Interestingly, the human DAP10 and DAP12 genes lie

adjacent on chromosome 19q13.1, in opposite transcriptional orientation and separated by only approximately 130 base pairs, presumably as a result of gene duplication. One unique feature of DAP10 is its short, but conserved, cytoplasmic tail which, contains a YxxM signaling motif, a potential src-homology 2 (SH2) domain-binding site for the p85 regulatory subunit of the phosphatidylinositol 3-kinase (PI 3-kinase). The physical and functional association of various OX2RH with DAP12 or DAP10 can be determined.

XII. Genetic analysis, animal studies

The sequences make available information and reagents useful for determination of the chromosomal mapping, disease marker correlation, and isolation and determination of the genetic structure of the respective genes. Intron/exon structure will be determined, and transgenic and deletion animals will be prepared. See, e.g., Goodnow (1992) "Transgenic Animals" in Roitt (ed.) Encyclopedia of Immunology, Academic Press, San Diego, pp. 1502-1504; Travis (1992) Science 256:1392-1394; Kuhn, et al. (1991) Science 254:707-710; Capecchi (1989) Science 244:1288; Robertson (ed. 1987) Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, IRL Press, Oxford; Rosenberg (1992) J. Clinical Oncology 10:180-199; and Cournoyer and Caskey (1993) Ann. Rev. Immunol. 11:297-329.

To determine function of OX2-OX2R interaction in vitro and in vivo, an adenovirus construct was prepared that produced in soluble form, the extracellular region of OX2RH1 fused to human IgG. A control construct was prepared that produced in soluble form, only the human IgG backbone. In the first instance, supernatants containing these fusion proteins were produced by cellular infection in vitro to test whether the OX2R fusion protein had biological function on the basis of binding to normally expressed mouse OX2. In the first series of studies, tissue sections of mouse spleen from normal as well as OX2-gene knockout (KO) mice, were prepared and OX2R, or control fusion proteins were added, and these reagents detected by addition of

an antibody binding to the human Fc portion of the fusion protein, and subsequent immunoperoxidase staining procedures to reveal binding. Weak binding only of the OX2R fusion protein, and only in normal but not OX2 KO mice, was detected on
 5 follicular dendritic cells and endothelial cells. Both cell types are known to express very high levels of the ligand OX2. Thus, the reagent bound to a physiological form of OX2 and no binding was observed in spleen where the ligand OX2 was absent due to gene targeting.

10 B cells are known to express the ligand OX2, but at lower level than on follicular dendritic cells and endothelial cells. Immunohistochemistry is not a particularly sensitive technique. Thus in a second study, the same fusion proteins were applied to isolated splenic leukocytes from normal and OX2 KO mouse and
 15 binding to B cells determined by flow cytometric analysis using mAb specific to the B220 molecule on B cells, and the more sensitive detection of the fusion proteins by secondary antibodies to human IgG coupled to phycoerythrin. In this case, all B cells in normal mice were labeled by the OX2R fusion
 20 protein but not by the control fusion protein. The interaction of the OX2R fusion protein with B cells was blocked by addition of a mAb called OX90 that is known to bind to the part of the mouse OX2 molecule that interacts with the OX2R. In addition, no binding above the background level seen with the control fusion
 25 protein was observed when OX2R fusion protein was added to B cells from OX2 KO mice.

The conclusions of these studies were: (1) that the OX2R fusion protein was biologically active and bound to OX2 on hematopoietic and non-hematopoietic cells; and (2) that the major
 30 ligand bound by OX2R is indeed the identified ligand OX2, on the basis of anti-OX2 (OX90) binding inhibition and lack of detectable binding to B cells from OX2 KO mice. These data cannot exclude the possibility that there are other ligands bound by the OX2R in addition to the known ligand OX2, as even flow
 35 cytometry had not detected cell-surface molecules expressed at very low level.

Transgenic mice can be generated by standard methods. Such animals are useful to determine the effects of deletion of the gene, in specific tissues, or completely throughout the organism. Such may provide interesting insight into development of the animal or particular tissues in various stages. Moreover, the effect on various responses to biological stress can be evaluated. See, e.g., Hogan, et al. (1995) Manipulating the Mouse Embryo: A Laboratory Manual (2d ed.) Cold Spring Harbor Laboratory Press. Likewise, deletion mice, e.g., knock out mice may be generated.

These animals will be subject to animal models to study the function of the genes in vivo. See, e.g., Gorczynski, et al. (1999) J. Immunol. 163:1654-1660; Mankoo, et al. (1999) Nature 400:69-73; Gorczynski, et al. (1999) Transplant. Proc. 31:577-578; and Gorczynski L, et al. (1999) J. Immunol. 162:774-781. Of particular interest will be the roles of macrophages or other myeloid cell populations, e.g., in the blood, lymphoid tissues, or solid organs, including the microglia in the nervous system. Tests of susceptibility to infection, autoimmune inflammation, and neural degeneration are indicated. Both antagonist and agonists will be useful reagents in the in vitro or in vivo models described or made available.

XIII. Screening for Substances Likely to Have Therapeutic Value

The biological effect of the OX2R/OX2 interaction can be investigated by using antibody reactive with OX2R (an experimental substitute for OX2) to crosslink OX2R molecules in the macrophage cell surface membrane, and looking for changes in, e.g., nitric oxide production or phosphorylation of signaling proteins.

The effects of perturbing the OX2R/OX2 interaction can be tested using macrophages carrying OX2R, by exposing them to a cross-linking binding partner such as a mAb for OX2R (e.g., OX102) or a recombinant multivalent version of OX2 in the presence and absence of the candidate substance. Comparing activity of the macrophages (e.g., nitric oxide production or

phosphorylation of signalling proteins) in the presence or absence of the candidate compound will indicate whether the candidate substance has a modulatory effect (e.g., inhibition or enhancement).

5 Candidate substances can also be tested in well established models of diseases in which macrophages are involved in the pathology of the disease, such as autoimmunity. For instance, established models such as experimental allergic encephalomyelitis can be used, as described and exemplified
10 above, e.g., with the models exhibiting accelerated onset of the autoimmune disease experimental autoimmune encephalomyelitis (EAE) in mice. OX2R mimetics may be advantageous in chronic conditions such as chronic granulomatosis. Combinations of agonists or antagonists of the OX2/OX2R signaling may be combined
15 with existing therapeutics for such conditions. See Physicians' Desk Reference Medical Economics Co, Montvale, NJ.

Analyses such as the above will indicate ways in which perturbation of the OX2R/OX2 interaction may be therapeutically beneficial. For example, the invention provides the means to
20 make recombinant versions of OX2RH which can be used in conjunction with the available OX2 proteins to screen for possible pharmacological reagents which block the interaction. The availability of interacting proteins provides the means for high throughput small molecule screening programs, as are used in
25 the pharmacology industry. See, e.g., meetings on High Throughput Screening, International Business Communications, Southborough, MA 01772-1749. Interactions through the cytoplasmic region of OX2RH are likely therapeutic targets and knowledge of the sequence and its interactions provide a means to
30 develop pharmacological reagents through similar screening methods.

XIV. Structure activity relationship

Information on the criticality of particular residues is
35 determined using standard procedures and analysis. Standard mutagenesis analysis is performed, e.g., by generating many

different variants at determined positions, e.g., at the positions identified above, and evaluating biological activities of the variants. This may be performed to the extent of determining positions which modify activity, or to focus on specific positions to determine the residues which can be substituted to either retain, block, or modulate biological activity.

Alternatively, analysis of natural variants can indicate what positions tolerate natural mutations. This may result from populational analysis of variation among individuals, or across strains or species. Samples from selected individuals are analyzed, e.g., by PCR analysis and sequencing. This allows evaluation of population polymorphisms.

All citations herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.

WHAT IS CLAIMED IS:

1. A composition of matter selected from:
 - a1) a substantially pure or recombinant polypeptide
5 comprising at least three distinct nonoverlapping
segments of at least four amino acids identical to
segments of SEQ ID NO: 2;
 - a2) a substantially pure or recombinant polypeptide
10 comprising at least two distinct nonoverlapping
segments of at least five amino acids identical to
segments of SEQ ID NO: 2;
 - a3) a natural sequence rodent OX2RH1 polypeptide comprising
mature SEQ ID NO: 2;
 - a4) a fusion polypeptide comprising rat OX2RH1 sequence;
 - 15 b1) a substantially pure or recombinant polypeptide
comprising at least three distinct nonoverlapping
segments of at least four amino acids identical to
segments of SEQ ID NO: 4;
 - b2) a substantially pure or recombinant polypeptide
20 comprising at least two distinct nonoverlapping
segments of at least five amino acids identical to
segments of SEQ ID NO: 4;
 - b3) a natural sequence rodent OX2RH1 polypeptide comprising
mature SEQ ID NO: 4;
 - 25 b4) a fusion polypeptide comprising human OX2RH1 sequence;
 - c1) a substantially pure or recombinant polypeptide
comprising at least three distinct nonoverlapping
segments of at least four amino acids identical to
segments of SEQ ID NO: 6;
 - 30 c2) a substantially pure or recombinant polypeptide
comprising at least two distinct nonoverlapping
segments of at least five amino acids identical to
segments of SEQ ID NO: 6;
 - c3) a natural sequence rodent OX2RH1 polypeptide comprising
35 mature SEQ ID NO: 6;
 - c4) a fusion polypeptide comprising mouse OX2RH1 sequence;

- d1) a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 8;
- 5 d2) a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 8;
- d3) a natural sequence rodent OX2RH1 polypeptide comprising mature SEQ ID NO: 8;
- 10 d4) a fusion polypeptide comprising human OX2RH2 sequence;
- e1) a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 10;
- 15 e2) a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 10;
- 20 e3) a natural sequence rodent OX2RH2 polypeptide comprising mature SEQ ID NO: 10;
- e4) a fusion polypeptide comprising mouse OX2RH2 sequence;
- f1) a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 12;
- 25 f2) a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 12;
- 30 f3) a natural sequence rodent OX2RH3 comprising mature SEQ ID NO: 12;
- f4) a fusion polypeptide comprising mouse OX2RH3 sequence;
- 35 g1) a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping

segments of at least four amino acids identical to
segments of SEQ ID NO: 20;

g2) a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 20;

g3) a natural sequence primate OX2RH1.2 polypeptide comprising mature SEQ ID NO: 20;

g4) a fusion polypeptide comprising primate OX2RH1.2 sequence;

h1) a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 23;

h2) a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 23;

h3) a natural sequence rodent OX2RH4 polypeptide comprising mature SEQ ID NO: 23; or

h4) a fusion polypeptide comprising mouse OX2RH4 sequence.

2. A binding compound comprising an antigen binding site from an antibody, which specifically binds to a natural OX2RH polypeptide of Claim 1, wherein:

- a) said binding compound is in a container;
- b) said OX2RH polypeptide is from a rodent or primate;
- c) said binding compound is an Fv, Fab, or Fab2 fragment;
- d) said binding compound is conjugated to another chemical moiety; or
- e) said antibody:
 - i) is raised against a peptide sequence of a mature polypeptide of Tables 1-3;
 - ii) is raised against a mature OX2RH;
 - iii) is raised to a purified mammalian OX2RH;
 - iv) is immunoselected;

- v) is a polyclonal antibody;
- vi) binds to a denatured OX2RH;
- vii) exhibits a Kd to antigen of at least 30 μM ;
- viii) is attached to a solid substrate, including a
5 bead or plastic membrane;
- ix) is in a sterile composition; or
- x) is detectably labeled, including a fluorescent
label.

10 3. An isolated or recombinant nucleic acid encoding said
OX2RH polypeptide of Claim 1, wherein said:

- a) OX2RH is from a mammal; or
- b) said nucleic acid:
 - i) encodes an antigenic peptide sequence of Tables 1-
15 3;
 - ii) encodes a plurality of antigenic peptide sequences
of Tables 1-3;
 - iii) exhibits identity over at least thirteen
nucleotides to a natural cDNA encoding said
20 segment;
 - iv) is an expression vector;
 - v) further comprises an origin of replication;
 - vi) is from a natural source;
 - vii) comprises a detectable label;
 - viii) comprises synthetic nucleotide sequence;
 - 25 ix) is less than 6 kb, preferably less than 3 kb;
 - x) is from a primate or rodent;
 - xi) comprises a natural full length coding sequence;
 - xii) is a hybridization probe for a gene encoding said
30 OX2RH;
 - xiii) further encodes DAP12 or DAP10; or
 - xiv) is a PCR primer, PCR product, or mutagenesis
primer.

35 4. A nucleic acid which:

- a) hybridizes under wash conditions of 30 minutes at 40° C and less than 2M salt to the coding portion of SEQ ID NO: 1, 3, 5, 7, 9, 11, 19, or 22; or
- b) exhibits identity over a stretch of at least about 30 nucleotides to a primate or rodent OX2RH cDNA.

5. A method of modulating physiology or development of a cell or tissue culture cells comprising contacting said cell with an agonist or antagonist of a mammalian OX2RH.

6. The method of Claim 5, wherein said:

- a) modulating physiology is:
 - i) enhancing meloid function; or
 - ii) enhancing immunity;
- b) agonist or antagonist attenuates OX2 mediated signaling to said cell; or
- c) said antagonist is:
 - i) an antibody to said OX2RH;
 - ii) a soluble OX2RH construct;
 - iii) a soluble OX2RH-Ig fusion; or
 - iv) an OX2R antisense nucleic acid.

7. The method of Claim 6, wherein:

- a) said modulating of physiology is enhancement of meloid cell function in vitro, and said antagonist is an OX2 mutein; or
- b) said modulating of physiology is enhancement of immunity in an animal being systemically treated with said antagonist.

8. A method for identification of a non-OX2 ligand for an OX2R, said method comprising screening a library of genes from an OX2 knock out mouse for binding to an OX2R-Ig fusion protein, and identifying genes which bind to said fusion protein.

MAMMALIAN PROTEINS; RELATED REAGENTS AND METHODS

5

ABSTRACT

Nucleic acids encoding mammalian, e.g., primate,
receptors, purified proteins and fragments thereof.
Antibodies, both polyclonal and monoclonal, are also provided.
10 Methods of using the compositions for both diagnostic and
therapeutic utilities are described.

**DECLARATION AND POWER OF
ATTORNEY FOR PATENT APPLICATION**

Attorney's Docket No. **DX01052K1**

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MAMMALIAN PROTEINS; RELATED REAGENTS AND METHODS

the specification of which

☐ is attached hereto.

☐ was filed on _____ as Application Serial No. _____

and was amended on _____ (if applicable).

☐ was filed on _____ as PCT International Application No. _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s):	Priority Claimed
<u>9911123.9</u> (Number)	<u>Great Britain</u> (Country)
<u>13 May 1999</u> (Day/Month/Year Filed)	<u>Yes or No</u>

Prior Foreign Application(s):	Priority Claimed
<u>9925989.7</u> (Number)	<u>Great Britain</u> (Country)
<u>03 November 1999</u> (Day/Month/Year Filed)	<u>Yes or No</u>

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

(Application Number)	(Filing Date)
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I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this

application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)

(Filing Date)

(Status – patented, pending, abandoned)

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Date <u>26 May 2000</u>	Date <u>8th June 2000</u>	Date <u>29th June 2000</u>
Signature of Fourth Inventor <i>[Signature]</i>	Signature of Fifth Inventor <i>G. J. Wyle</i>	Signature of Sixth Inventor <i>Holcy Chuminski</i>
Date <u>7/20/00</u>	Date <u>8/6/2000</u>	Date <u>29th June 2000</u>
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Date <u>30th June 2000</u>	Date <u>29/6/2000</u>	Date <u>29th June 2000</u>



PTO/PCT Rec'd 04 SEP 2002

#5

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Sedgwick, Jonathon D.

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<140> 10/009,445

<141> 2000-05-11

<150> PCT/US00/12998

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Ser Trp Pro Val Lys Met Ala Thr Asn Ala Val Leu Cys Cys Pro Pro

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Ile

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Asp Gly Asn Phe His Arg Gly Tyr His Leu Gln Val Leu Val Thr Pro
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Val Ala Gly Lys Pro Ala Ala His Ile Ser Trp Ile Pro Glu Gly Asp
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Val Ser His Leu Thr Gly Asn Lys Ser Leu Tyr Ile Glu Leu Leu Pro
245 250 255

Val Pro Gly Ala Lys Lys Ile Ser Lys Ile Ile Tyr Ser Ile Tyr His
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Ile Trp Gly Val Phe Val Ala Gly Ser Ser Cys Thr Asp Lys Asn Gln

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Thr Thr Gln Asn Asn Ser Ser Ser Pro Leu Thr Gln Val Asn Thr Thr

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gtg tct gta cag ata ggt aca aag gct ctg ctc tgc tgc ttt tct att
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Val Ser Val Gln Ile Gly Thr Lys Ala Leu Leu Cys Cys Phe Ser Ile

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120

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Thr Lys Ala Val Leu Ile Thr Trp Ile Ile Lys Leu Arg Gly Leu Pro
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Ser Cys Thr Ile Ala Tyr Lys Val Asp Thr Lys Thr Asn Glu Thr Ser
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Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro Asp His Ser Pro
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Gln Val Leu Val Pro Pro Glu Val Thr Tyr Phe Pro Glu Lys Asn Arg
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Ser Ala Val Cys Glu Ala Met Ala Gly Lys Pro Ala Ala Gln Ile Ser
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Pro Tyr Ile Ile Pro Ser Ile Ile Ile Leu Ile Ile Ile Gly Cys Ile
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Cys Leu Leu Lys Ile Ser Gly Phe Arg Lys Cys Lys Leu Pro Lys Leu
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Cys Pro Pro Ile Ala Leu Arg Asn Leu Ile Ile Ile Thr Trp Glu Ile

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Asn Glu Thr Lys Glu Thr Asn Cys Thr Val Glu Arg Ile Thr Trp Val

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90

95

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Thr Lys Gln Glu Tyr Trp Gly Asn Gly Thr Val Thr Val Lys Ser Thr
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Thr Ser Gly Ser Pro Ala Leu Ser Leu Leu Ile Ile Leu Tyr Val Lys
210 215 220

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Asp His Ile Pro Asp Leu Gln Ile Ser Ala Val Ala Leu Gln His Glu
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Arg Thr

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52

atggggacaa attgtggagc atagaccaa agaaaggcca tccagagact gccccaccta 8
12

aggacccatc ccatatacag acaccaaacc cagacactac tgaagatgct gcgaagcggt 8
72

tgctgacagg agcctgttat agctgtctcc tgagaggctc agccagagcc tgacaaatac 9
32

ataggtagat gcttgcagcc aacaactgga ctgagcaaaa aatctccatt ggaggagtta 9
92

gagaaaggac tgaagagggt gaaagggttt gcagcccat aggaagaaca acaatatcaa 10
52

ccaaccagat ctcccagagc tcccaggac taa 10
85

<210> 10
<211> 194
<212> PRT
<213> mus musculus

<400> 10

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Arg Gly Gln Pro Ser Cys Ile Met Ala Tyr Lys Val Glu Thr Lys Glu
1 5 10 15

Thr Asn Glu Thr Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro
20 25 30

Asp His Ile Pro Asp Leu Gln Ile Ser Ala Val Ala Leu Gln His Glu
35 40 45

Gly Asn Tyr Leu Cys Glu Ile Thr Thr Pro Glu Gly Asn Phe His Lys
50 55 60

Val Tyr Asp Leu Gln Val Leu Val Pro Pro Glu Val Thr Tyr Phe Leu
65 70 75 80

Gly Glu Asn Arg Thr Ala Val Cys Glu Ala Met Ala Gly Lys Pro Ala
85 90 95

Ala Gln Ile Ser Trp Thr Pro Asp Gly Asp Cys Val Thr Lys Ser Glu
100 105 110

Ser His Ser Asn Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu
115 120 125

Gln Asn Asn Val Ser Ala Val Ser Cys Ile Val Ser His Ser Thr Gly
130 135 140

Asn Gln Ser Leu Ser Ile Glu Leu Ser Arg Gly Thr Thr Ser Thr Thr
145 150 155 160

Pro Ser Leu Leu Thr Ile Leu Tyr Val Lys Met Val Leu Leu Gly Ile
165 170 175

Ile Leu Leu Lys Val Gly Phe Ala Phe Phe Gln Lys Arg Asn Val Thr
180 185 190

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Arg Thr

<210> 11
 <211> 1354
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (42)..(875)
 <223>

<400> 11
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 56

Met His Ala Leu Gly

1 5

agg act ctg gct ttg atg tta ctc atc ttc atc act att ttg gtg cct 1
 04
 Arg Thr Leu Ala Leu Met Leu Leu Ile Phe Ile Thr Ile Leu Val Pro

10 15 20

gag tca agt tgt tca gtg aaa gga cgg gag gag atc cca ccg gat gat 1
 52
 Glu Ser Ser Cys Ser Val Lys Gly Arg Glu Glu Ile Pro Pro Asp Asp

25 30 35

tca ttt cct ttt tca gat gat aat atc ttc cct gat gga gtg ggc gtc 2
 00
 Ser Phe Pro Phe Ser Asp Asp Asn Ile Phe Pro Asp Gly Val Gly Val

40 45 50

acc atg gag att gag att atc act cca gtg tct gta cag ata ggt atc 2
 48
 Thr Met Glu Ile Glu Ile Ile Thr Pro Val Ser Val Gln Ile Gly Ile

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55

60

65

aag gct cag ctt ttc tgt cat cct agt cca tca aaa gaa gca aca ctt 2
 96
 Lys Ala Gln Leu Phe Cys His Pro Ser Pro Ser Lys Glu Ala Thr Leu
 70 75 80 85

aga ata tgg gaa ata act ccc aga gac tgg cct tcc tgc aga cta ccc 3
 44
 Arg Ile Trp Glu Ile Thr Pro Arg Asp Trp Pro Ser Cys Arg Leu Pro
 90 95 100

tac aga gca gag ttg cag cag atc agt aaa aaa atc tgt act gag aga 3
 92
 Tyr Arg Ala Glu Leu Gln Gln Ile Ser Lys Lys Ile Cys Thr Glu Arg
 105 110 115

gga acc act agg gtc cct gca cat cac cag agt tct gac ctt ccc atc 4
 40
 Gly Thr Thr Arg Val Pro Ala His His Gln Ser Ser Asp Leu Pro Ile
 120 125 130

aaa tca atg gcc ctc aag cat gat ggg cat tac tca tgt cgg ata gaa 4
 88
 Lys Ser Met Ala Leu Lys His Asp Gly His Tyr Ser Cys Arg Ile Glu
 135 140 145

aca aca gat ggg att ttc caa gag aga cat agc atc caa gtg cca ggg 5
 36
 Thr Thr Asp Gly Ile Phe Gln Glu Arg His Ser Ile Gln Val Pro Gly
 150 155 160 165

gaa aat aga act gta gtt tgt gag gca att gca agc aag cct gct atg 5
 84
 Glu Asn Arg Thr Val Val Cys Glu Ala Ile Ala Ser Lys Pro Ala Met

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170

175

180

cag atc ttg tgg act cca gat gag gac tgt gtc act aag agt aaa tca
32
Gln Ile Leu Trp Thr Pro Asp Glu Asp Cys Val Thr Lys Ser Lys Ser

6

185

190

195

cac aat gac acc atg att gtc agg agc aag tgc cac agg gag aaa aac
80
His Asn Asp Thr Met Ile Val Arg Ser Lys Cys His Arg Glu Lys Asn

6

200

205

210

aat ggc cac agt gtg ttc tgc ttt atc tcc cat ttg act gat aac tgg
28
Asn Gly His Ser Val Phe Cys Phe Ile Ser His Leu Thr Asp Asn Trp

7

215

220

225

att ctc tcc atg gaa cag aat cga ggt aca acc agc atc ctg cct tcc
76
Ile Leu Ser Met Glu Gln Asn Arg Gly Thr Thr Ser Ile Leu Pro Ser

7

230

235

240

245

ttg ctg agc att ctc tat gtg aaa ctg gct gta act gtt ctc atc gta
24
Leu Leu Ser Ile Leu Tyr Val Lys Leu Ala Val Thr Val Leu Ile Val

8

250

255

260

gga ttt gct ttt ttc cag aag aga aat tat ttc aga gtg cca gaa ggc
72
Gly Phe Ala Phe Phe Gln Lys Arg Asn Tyr Phe Arg Val Pro Glu Gly

8

265

270

275

tcc tgaggagagt ggtctgtggt taagatgaga tttaccacca tctgaaagac
25

9

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Ser

```

atcttgtcta ccgcgcagcg tgctgagatt ccgagaagca gccacagaac ctactaggaa    9
85
gacaaatctg atgtggttgt caatcctttc aatggacctg agtacttcta taaaccogag    10
45
tgaggttgtg ctggacccag gagccaggct aggtcatata tgttgatttt tgctgcaaga    11
05
cctcatggtt tatctacaaa tcctaaattc ttctacttcc agtttttaaaa cttttggccc    11
65
aagcatttta tccacagcat aacaccttta aagaaactct cccacggaaa ctgctggttc    12
25
catggaatgg aaaattgcaa catggtttac aagacagtgc aaaccaagca gcattccaag    12
85
atatgagctt cagaaagtta caggaactgt cttgggacga gaaagaagga ttaaatagtt    13
45
cccagtcctc                                     13
54

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<210> 12
 <211> 278
 <212> PRT
 <213> Mus musculus

<400> 12

Met His Ala Leu Gly Arg Thr Leu Ala Leu Met Leu Leu Ile Phe Ile
 1 5 10 15

Thr Ile Leu Val Pro Glu Ser Ser Cys Ser Val Lys Gly Arg Glu Glu
 20 25 30

Ile Pro Pro Asp Asp Ser Phe Pro Phe Ser Asp Asp Asn Ile Phe Pro
 35 40 45

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Ser Ile Leu Pro Ser Leu Leu Ser Ile Leu Tyr Val Lys Leu Ala Val
245 250 255

Thr Val Leu Ile Val Gly Phe Ala Phe Phe Gln Lys Arg Asn Tyr Phe
260 265 270

Arg Val Pro Glu Gly Ser
275

<210> 13
<211> 981
<212> DNA
<213> rodent

<220>
<221> misc_feature
<222> (1)..(981)
<223> n may be a, c, g, or t.

<400> 13
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60

gcngarwsnw sntgyccnga yaaraaycar acnatgcara ayaaywsnws nacnatgacn 1
20

gargtnaaya cnacngtntt ygtncaratg ggnaaraarg cnytnytntg ytgyccnwsn 1
80

athwsnytna cnaargtnat hytnathacn tggacnatha cnytnmgngg ncarccnwsn 2
40

tgyathathw sntayaargc ngayacnmgn garacncayg arwsnaaytg ywsngaymgn 3
00

wsnathacnt gggcnwsnac nccngayytn gcncngayy tncarathws ngcngtngcn 3
60

ytnarcayg arggnmgnta ywsntgygay athgcngtnc cngayggnaa yttycaraay 4
20

athtaygayy tncargtnyt ngtnccnccn gargtnacnc aytttyccngg ngaraaymgn 4
80

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acngcngtnt gygargcnat hgcnggnaar ccngcngcnc arathwsntg gacnccngay 5
40

ggngaytgyg tngcnaaraa ygarwsncay wsnaayggna cngtnacngt nmgnwsnacd 6
00

tgycaytggg arcarwsnca ygtnwsngtn gtnattygyg tngtnwsnca yytnacnacn 6
60

ggnaaycarw snytnwsnat hgarytnggn mgnggnggng aycarytnyt nggnwsntay 7
20

athcartaya thathccnws nathathath ytnathatha thggntgyat htgyytnytn 7
80

aarathwsng gntgymgnaa rtgyaarytn ccnaarwsng gngcnacncc ngayathgar 8
40

gargaygara tgcarccnta ygcnwsntay acngaraarw snaayccnyt ntaygayacn 9
00

gtnacnacna cngargcnca yccngcnwsn carggnaarg tnaayggnac ngaytgyytn 9
60

acnytnwsng cnatgggnat h 9
81

<210> 14
<211> 885
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)..(885)
<223> N may be a, c, g, or t.

<400> 14
atgytntgyc cntggmgnac ngcnaayytn ggnytnytny tnathytnac nathttyytn
60

gtngcngarg cngarggngc ngcncarccn aayaaywsny tnatgytnca racnwsnaar 1
20

garaaycayg cnytngcnws nwsnwsnytn tgyatggayg araarcarat hacncaraay 1

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80

taywsnaarg tnytnngcnga rgtnaayacn wsntggccng tnaaratggc nacnaaygcn 2
40

gtnytntgyt gyccnccnat hgcnymnmg n aayytnatha thathacntg ggarathath 3
00

ytnmgnggnc arccnwsntg yacnaargcn tayaaraarg aracnaayga racnaargar 3
60

acnaaytgga cngaygarmg nathacntgg gtnwsnmgnc cngaycaraa ywsngayytn 4
20

carathmgna cngtnngcnat hacncaygay ggntaytaym gntgyathat ggtnacnccn 4
80

gayggnaayt tycaymgngg ntaycayytn cargtnytng tnacnccnga rgtnacnytn 5
40

ttycaraaym gnaaymgnc ngcngtntgy aargcngtng cnggnaarcc ngcngcncay 6
00

athwsntgga thcngargg ngaytgygcn acnaarcarg artaytggws naayggncn 6
60

gtnacngtna arwsnacntg ycaytgggar gtnacayaayg tnwsnacngt nacntgygay 7
20

gtnwsncayy tnacnggnaa yaarwsnytn tayathgary tnytnccngt nccnggngcn 7
80

aaraarathw snaarathat htaywsnath taycayccnt aytaytayta yytngaygay 8
40

mgnggnathc ayytngtngt ngarwsncar tggytncara arath 8
85

<210> 15
<211> 978
<212> DNA
<213> rodent

<220>
<221> misc_feature
<222> (1)..(978)
<223> n may be a, c, g, or t.

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acnaargtng argcnttycc ngtnwsncar ggngargtna ayggnacnga ytgyytnacn 9
60

ytnwsngcna thggnath 9
78

<210> 16
<211> 750
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)..(750)
<223> N may be a, c, g, or t.

<400> 16
atgggnggna arcaratgac ncaraaytay wsnacnatht tygcngargg naayathwsn
60

carccngtny tnatggayat haaygcngtn ytntgytgyc cnccnathgc nytnmgnaay 1
20

ytnathatha thacntggga rathathytn mgnggncarc cnwsntgyac naargcntay 1
80

aaraargara cnaaygarac naargaracn aaytgyacng tngarmgnat hacntgggtn 2
40

wsnmgncng aycaraayws ngayytncar athmgncng tngayacnac ncaygayggn 3
00

taytaymgng gnathgtngt nacncngay ggnaayttyc aymnggnta ycayytncar 3
60

gtnytngtna cncngargt naayytntty carwsnmgna ayathacngc ngtnntgyaar 4
20

gcngtnacng gnaarccngc ngcncarath wsntggathc cngarggnws nathytngcn 4
80

acnaarcarg artaytgggg naayggnacn gtnacngtna arwsnacntg yccntgggar 5
40

ggncayaarw snacngtnac ntgycaygtn wsncaaytna cnggnaayaa rwsnytnwsn 6
00

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gtnaarytna aywsnggnyt nmgnacnwsn ggnwsnccng cnytnwsnyt nytnathath 6
60

ytntaygtna arytnwsnyt nttygtngtn athytngtna cnaenggnntt ygtnttytty 7
20

carmgnatha aycaygtnmg naargtnytn 7
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<210> 17
<211> 582
<212> DNA
<213> rodent

<220>
<221> misc_feature
<222> (1)..(582)
<223> n may be a, c, g, or t.

<400> 17
mgnggncarc cnwsntgyat hatggcntay aargtngara cnaargarac naaygaracn
60

tgyytnggnm gnaayathac ntgggcnwsn acncngayc ayathccnga yytnicarath 1
20

wsngcngtng cnytnarca ygarggnaay tayytntgyg arathacnac nccngarggn 1
80

aayttycaya argtntayga yytnargtn ytngtncnc cngargtnac ntayttyytn 2
40

ggngaraaym gnacngcngt ntgygargcn atggcnggna arccngcngc ncarathwsn 3
00

tggacncng ayggngaytg ygtnacnaar wsngarwsnc aywsnaaygg nacngtnacn 3
60

gtnmgnwsna cntgycaytg ggarcaraay aaygtnwsng cngtnwsntg yathgtwnsn 4
20

caywsnacng gnaaycarws nytnwsnath garytnwsnm gnggnacnac nwsnacnac 4
80

ccnwsnytny tnacnathyt ntaygtnaar atggtnytny tnggnathat hytnytnaar 5

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40

gtnggnttyg cnttyttyca raarmgnaay gtnacnmgna cn
82

5

<210> 18
 <211> 834
 <212> DNA
 <213> rodent

<220>
 <221> misc_feature
 <222> (1)..(834)
 <223> n may be a, t, g, or c.

<400> 18
 atgcaygcn ytnngmgnac nytnngcnytn atgytnyt na thtttyathac nathytnngtn
 60

ccngarwsnw sntgywsngt naarggmgn gargarathc cncngayga ywsnttyccn
 20 1

ttywsngayg ayaayathtt yccngayggn gtnggngtna cnatggarat hgarathath
 80 1

acncngtnw sngtncarat hggnaathaar gcncarytnt tytgycaycc nwsncnwsn
 40 2

aargargcna cnytnmgna htgggarath acncnmgng aytggccnws ntgymgnytn
 00 3

ccntaymgng cngarytnca rcarathwsn aaraaratht gyacngarmg nggnacnacn
 60 3

mgngtnccng cncaycayca rwsnwsngay ytnccnatha arwsnatggc nytnaarcay
 20 4

gayggncayt aywsntgymg nathgaracn acngayggna thttycarga rmgncaaywsn
 80 4

athcargtn cngngararaa ymgnaengtn gtntgygarg cnathgcnws naarcngcn
 40 5

atgcarathy tntggacncc ngaygargay tgygtnacna arwsnaarws ncayaaygay
 00 6

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acnatgathg tnmgnwsnaa rtgycaymgn garaaraaya ayggncayws ngtnnttytgy 6
60

ttyathwsnc ayytnacnga yaaytggath ytnwsnatgg arcaraaymg nggnacnacn 7
20

wsnathytnc cnwsnytnyt nwsnathytn taygtnaary tngcngtnac ngtnytnath 7
80

gtnggnttyg cnttyttyca raarmgnaay tayttymgng tncngargg nwsn 8
34

<210> 19
<211> 1047
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1044)
<223>

<400> 19
atg ctc tgc cct tgg aga act gct aac cta ggg cta ctg ttg att ttg
48
Met Leu Cys Pro Trp Arg Thr Ala Asn Leu Gly Leu Leu Leu Ile Leu
1 5 10 15

act atc ttc tta gtg gcc gaa gcg gag ggt gct gct caa cca aac aac
96
Thr Ile Phe Leu Val Ala Glu Ala Glu Gly Ala Ala Gln Pro Asn Asn
20 25 30

tca tta atg ctg caa act agc aag gag aat cat gct tta gct tca agc 1
44
Ser Leu Met Leu Gln Thr Ser Lys Glu Asn His Ala Leu Ala Ser Ser
35 40 45

agt tta tgt atg gat gaa aaa cag att aca cag aac tac tcg aaa gta 1
92

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Ser Leu Cys Met Asp Glu Lys Gln Ile Thr Gln Asn Tyr Ser Lys Val

50

55

60

ctc gca gaa gtt aac act tca tgg cct gta aag atg gct aca aat gct
40
Leu Ala Glu Val Asn Thr Ser Trp Pro Val Lys Met Ala Thr Asn Ala

2

65

70

75

80

gtg ctt tgt tgc cct cct atc gca tta aga aat ttg atc ata ata aca
88
Val Leu Cys Cys Pro Pro Ile Ala Leu Arg Asn Leu Ile Ile Ile Thr

2

85

90

95

tgg gaa ata atc ctg aga ggc cag cct tcc tgc aca aaa gcc tac agg
36
Trp Glu Ile Ile Leu Arg Gly Gln Pro Ser Cys Thr Lys Ala Tyr Arg

3

100

105

110

aaa gaa aca aat gag acc aag gaa acc aac tgt act gat gag aga ata
84
Lys Glu Thr Asn Glu Thr Lys Glu Thr Asn Cys Thr Asp Glu Arg Ile

3

115

120

125

acc tgg gtc tcc aga cct gat cag aat tcg gac ctt cag att cgt cca
32
Thr Trp Val Ser Arg Pro Asp Gln Asn Ser Asp Leu Gln Ile Arg Pro

4

130

135

140

gtg gcc atc act cat gac ggg tat tac aga tgc ata atg gta aca cct
80
Val Ala Ile Thr His Asp Gly Tyr Tyr Arg Cys Ile Met Val Thr Pro

4

145

150

155

160

gat ggg aat ttc cat cgt gga tat cac ctc caa gtg tta gtt aca cct

5

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28

Asp Gly Asn Phe His Arg Gly Tyr His Leu Gln Val Leu Val Thr Pro

165

170

175

gaa gtg acc ctg ttt caa aac agg aat aga act gca gta tgc aag gca
76

Glu Val Thr Leu Phe Gln Asn Arg Asn Arg Thr Ala Val Cys Lys Ala

180

185

190

gtt gca ggg aag cca gct gcg cag atc tcc tgg atc cca gag ggc gat
24

Val Ala Gly Lys Pro Ala Ala Gln Ile Ser Trp Ile Pro Glu Gly Asp

195

200

205

tgt gcc act aag caa gaa tac tgg agc aat ggc aca gtg act gtt aag
72

Cys Ala Thr Lys Gln Glu Tyr Trp Ser Asn Gly Thr Val Thr Val Lys

210

215

220

agt aca tgc cac tgg gag gtc cac aat gtg tct acc gtg acc tgc cac
20

Ser Thr Cys His Trp Glu Val His Asn Val Ser Thr Val Thr Cys His

225

230

235

240

gtc tcc cat ttg act ggc aac aag agt ctg tac ata gag cta ctt cct
68

Val Ser His Leu Thr Gly Asn Lys Ser Leu Tyr Ile Glu Leu Leu Pro

245

250

255

gtt cca ggt gcc aaa aaa tca gca aaa tta tat att cca tat atc atc
16

Val Pro Gly Ala Lys Lys Ser Ala Lys Leu Tyr Ile Pro Tyr Ile Ile

260

265

270

DX01052K1.ST25

ctt act att att att ttg acc atc gtg gga ttc att tgg ttg ttg aaa 8
64

Leu Thr Ile Ile Ile Leu Thr Ile Val Gly Phe Ile Trp Leu Leu Lys

275

280

285

gtc aat ggc tgc aga aaa tat aaa ttg aat aaa aca gaa tct act cca 9
12

Val Asn Gly Cys Arg Lys Tyr Lys Leu Asn Lys Thr Glu Ser Thr Pro

290

295

300

gtt gtt gag gag gat gaa atg cag ccc tat gcc agc tac aca gag aag 9
60

Val Val Glu Glu Asp Glu Met Gln Pro Tyr Ala Ser Tyr Thr Glu Lys

305

310

315

320

aac aat cct ctc tat gat act aca aac aag gtg aag gca tct cag gca 10
08

Asn Asn Pro Leu Tyr Asp Thr Thr Asn Lys Val Lys Ala Ser Gln Ala

325

330

335

tta caa agt gaa gtt gac aca gac ctc cat act tta taa 10
47

Leu Gln Ser Glu Val Asp Thr Asp Leu His Thr Leu

340

345

<210> 20

<211> 348

<212> PRT

<213> Homo sapiens

<400> 20

Met Leu Cys Pro Trp Arg Thr Ala Asn Leu Gly Leu Leu Leu Ile Leu
1 5 10 15

Thr Ile Phe Leu Val Ala Glu Ala Glu Gly Ala Ala Gln Pro Asn Asn

DX01052K1.ST25

20

25

30

Ser Leu Met Leu Gln Thr Ser Lys Glu Asn His Ala Leu Ala Ser Ser
35 40 45

Ser Leu Cys Met Asp Glu Lys Gln Ile Thr Gln Asn Tyr Ser Lys Val
50 55 60

Leu Ala Glu Val Asn Thr Ser Trp Pro Val Lys Met Ala Thr Asn Ala
65 70 75 80

Val Leu Cys Cys Pro Pro Ile Ala Leu Arg Asn Leu Ile Ile Ile Thr
85 90 95

Trp Glu Ile Ile Leu Arg Gly Gln Pro Ser Cys Thr Lys Ala Tyr Arg
100 105 110

Lys Glu Thr Asn Glu Thr Lys Glu Thr Asn Cys Thr Asp Glu Arg Ile
115 120 125

Thr Trp Val Ser Arg Pro Asp Gln Asn Ser Asp Leu Gln Ile Arg Pro
130 135 140

Val	Ala	Ile	Thr	His	Asp	Gly	Tyr	Tyr	Arg	Cys	Ile	Met	Val	Thr	Pro
145					150					155					160

Asp Gly Asn Phe His Arg Gly Tyr His Leu Gln Val Leu Val Thr Pro
165 170 175

Glu Val Thr Leu Phe Gln Asn Arg Asn Arg Thr Ala Val Cys Lys Ala
180 185 190

Val Ala Gly Lys Pro Ala Ala Gln Ile Ser Trp Ile Pro Glu Gly Asp
195 200 205

Cys Ala Thr Lys Gln Glu Tyr Trp Ser Asn Gly Thr Val Thr Val Lys

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210

215

220

Ser Thr Cys His Trp Glu Val His Asn Val Ser Thr Val Thr Cys His
225 230 235 240

Val Ser His Leu Thr Gly Asn Lys Ser Leu Tyr Ile Glu Leu Leu Pro
245 250 255

Val Pro Gly Ala Lys Lys Ser Ala Lys Leu Tyr Ile Pro Tyr Ile Ile
260 265 270

Leu Thr Ile Ile Ile Leu Thr Ile Val Gly Phe Ile Trp Leu Leu Lys
275 280 285

Val Asn Gly Cys Arg Lys Tyr Lys Leu Asn Lys Thr Glu Ser Thr Pro
290 295 300

Val Val Glu Glu Asp Glu Met Gln Pro Tyr Ala Ser Tyr Thr Glu Lys
305 310 315 320

Asn Asn Pro Leu Tyr Asp Thr Thr Asn Lys Val Lys Ala Ser Gln Ala
325 330 335

Leu Gln Ser Glu Val Asp Thr Asp Leu His Thr Leu
340 345

<210> 21
<211> 1044
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (1)..(1044)
<223> n may be a, t, g, or c.

<400> 21
atgytntgyc cntggmgnac ngcnaaytn ggnytnytny tnathytnac nathttytn

DX01052K1.ST25

60

gtngcngarg cngarggngc ngcnarccn aayaaywsny tnatgytnca racnwsnaar 20	1
garaaycayg cnytnngcnws nwsnwsnytn tgyatggayg araarcarat hacncaraay 80	1
taywsnaarg tnytnngcnga rgtnaayacn wsntggccng tnaaratggc nacnaaygen 40	2
gtnytntgyt gyccnccnat hgcnymnmg aayytnatha thathacntg ggarathath 00	3
ytnmgnggnc arccnwsntg yacnaargcn taymgnaarg aracnaayga racnaargar 60	3
acnaaytgya cngaygarmg nathacntgg gtnwsnmgnc cngaycaraa ywsngayytn 20	4
carathmgnc cngtnngcnat hacncaygay ggntaytaym gntgyathat ggtnacnccn 80	4
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athwsntgga thccngargg ngaytgygcn acnaarcarg artaytggws naayggnaen 60	6
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garwsnacnc cngtnngtna rgargaygar atgcarccnt aygcnwsnta yacngaraar 60	9
aayaayccny tntaygayac nacnaayaar gtnaargcnw sncargcnytn ncarwsngar 00	10

DX01052K1.ST25

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aat att ttt gtg tct ggg tca agt tgt act gat gag aat caa aca ata
96
Asn Ile Phe Val Ser Gly Ser Ser Cys Thr Asp Glu Asn Gln Thr Ile
20 25 30

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Gln Asn Asp Ser Ser Ser Ser Leu Thr Gln Val Asn Thr Thr Met Ser
35 40 45

gta cag atg gat aaa aag gct ctg ctc tgc tgc ttt tct agt cca ctg 1
92
Val Gln Met Asp Lys Lys Ala Leu Leu Cys Cys Phe Ser Ser Pro Leu
50 55 60

ata aat gca gta tta atc aca tgg ata ata aaa cac aga cac ctg cct 2
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Ile Asn Ala Val Leu Ile Thr Trp Ile Ile Lys His Arg His Leu Pro

65 70 75 80

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88
Ser Cys Thr Ile Ala Tyr Asn Leu Asp Lys Lys Thr Asn Glu Thr Ser

85 90 95

tgc ttg ggc agg aac atc acc tgg gcc tcc aca cct gac cac agt cct 3
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Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro Asp His Ser Pro

100 105 110

gaa ctt cag atc agt gca gtg gcc ctc cag cat gag ggg act tac aca 3
84
Glu Leu Gln Ile Ser Ala Val Ala Leu Gln His Glu Gly Thr Tyr Thr

115 120 125

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32
Cys Glu Ile Val Thr Pro Glu Gly Asn Leu Glu Lys Val Tyr Asp Leu

130 135 140

caa gtg ctg gtg ccc cct gag gta acc tac ttt cca ggg aaa aac aga 4
80
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145 150 155 160

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165 170 175

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190

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Ser Val Val Ser Cys Leu Val Ser His Ser Thr Gly Asn Gln Ser Leu

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7

Ser Ile Glu Leu Ser Gln Gly Thr Met Thr Thr Pro Arg Ser Leu Leu

240

7

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Ser	Cys	Thr	Ile	Ala	Tyr	Asn	Leu	Asp	Lys	Lys	Thr	Asn	Glu	Thr	Ser
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Cys	Glu	Ile	Val	Thr	Pro	Glu	Gly	Asn	Leu	Glu	Lys	Val	Tyr	Asp	Leu
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145					150					155					160
Thr	Ala	Val	Cys	Glu	Ala	Met	Ala	Gly	Lys	Pro	Ala	Ala	Gln	Ile	Ser
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Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu Gln Asn Asn Val
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Ser Val Val Ser Cys Leu Val Ser His Ser Thr Gly Asn Gln Ser Leu
210 215 220

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225 230 235 240

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acncargtna ayacnacnat gwsngtnicar atggayaara argcnytnyt ntgytgytty 1
80

wsnwsnccny tnathaaygc ngtnytnath acntggatha thaarcaymg ncayytnccn 2
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wsntgyacna thgcntayaa yytngaayaar aaracnaayg aracnwsntg yytnggnmgn 3
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SEQUENCE SUBMISSION

SEQ ID NO: 1 is rodent OX2R nucleotide sequence.
SEQ ID NO: 2 is rodent OX2R polypeptide sequence.
SEQ ID NO: 3 is primate OX2R homolog 1 nucleotide sequence.
SEQ ID NO: 4 is primate OX2R homolog 1 polypeptide sequence.
SEQ ID NO: 5 is rodent OX2R homolog 1 nucleotide sequence.
SEQ ID NO: 6 is rodent OX2R homolog 1 polypeptide sequence.
SEQ ID NO: 7 is primate OX2R homolog 2 nucleic acid sequence.
SEQ ID NO: 8 is primate OX2R homolog 2 polypeptide sequence.
SEQ ID NO: 9 is rodent OX2R homolog 2 nucleic acid sequence.
SEQ ID NO: 10 is rodent OX2R homolog 2 polypeptide sequence.
SEQ ID NO: 11 is rodent OX2R homolog 3 nucleic acid sequence.
SEQ ID NO: 12 is rodent OX2R homolog 3 polypeptide sequence.
SEQ ID NO: 13 is rodent OX2R polypeptide encoding sequence.
SEQ ID NO: 14 is primate OX2R homolog 1 polypeptide encoding sequence.
SEQ ID NO: 15 is rodent OX2R homolog 1 polypeptide encoding sequence.
SEQ ID NO: 16 is primate OX2R homolog 2 polypeptide encoding sequence.
SEQ ID NO: 17 is rodent OX2R homolog 2 polypeptide encoding sequence.
SEQ ID NO: 18 is rodent OX2R homolog 3 polypeptide encoding sequence.
SEQ ID NO: 19 is primate OX2R homolog 1.2 nucleotide sequence.
SEQ ID NO: 20 is primate OX2R homolog 1.2 polypeptide sequence.
SEQ ID NO: 21 is primate OX2R homolog 1.2 polypeptide encoding sequence.
SEQ ID NO: 22 is rodent OX2R homolog 4 nucleic acid sequence.
SEQ ID NO: 23 is rodent OX2R homolog 4 polypeptide sequence.
SEQ ID NO: 24 is rodent OX2R homolog 4 polypeptide encoding sequence.

<110> Medical Research Council
Schering Corporation

<120> Mammalian Proteins; Related Reagents and Methods

<130> DX01052K1 PCT

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25 30 35 40
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Cys Ile Ile Ser Tyr Lys Ala Asp Thr Arg Glu Thr His Glu Ser Asn
60 65 70
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75 80 85
Asp Leu Gln Ile Ser Ala Val Ala Leu Gln His Glu Gly Arg Tyr Ser
90 95 100
Cys Asp Ile Ala Val Pro Asp Gly Asn Phe Gln Asn Ile Tyr Asp Leu
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Gln Val Leu Val Pro Pro Glu Val Thr His Phe Pro Gly Glu Asn Arg
125 130 135
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140 145 150
Trp Thr Pro Asp Gly Asp Cys Val Ala Lys Asn Glu Ser His Ser Asn
155 160 165
Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu Gln Ser His Val
170 175 180
Ser Val Val Phe Cys Val Val Ser His Leu Thr Thr Gly Asn Gln Ser
185 190 195 200
Leu Ser Ile Glu Leu Gly Arg Gly Gly Asp Gln Leu Leu Gly Ser Tyr
205 210 215
Ile Gln Tyr Ile Ile Pro Ser Ile Ile Ile Leu Ile Ile Ile Gly Cys
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Ile Cys Leu Leu Lys Ile Ser Gly Cys Arg Lys Cys Lys Leu Pro Lys
235 240 245
Ser Gly Ala Thr Pro Asp Ile Glu Glu Asp Glu Met Gln Pro Tyr Ala
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Ser Tyr Thr Glu Lys Ser Asn Pro Leu Tyr Asp Thr Val Thr Thr Thr
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Met Leu Cys Pro Trp Arg
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Ser Lys Glu Asn His Ala Leu Ala Ser Ser Ser Leu Cys Met Asp Glu
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aaa cag att aca cag aac tac tcg aaa gta ctc gca gaa gtt aac act 426
Lys Gln Ile Thr Gln Asn Tyr Ser Lys Val Leu Ala Glu Val Asn Thr
30 35 40
tca tgg cct gta aag atg gct aca aat gct gtg ctt tgt tgc cct cct 474
Ser Trp Pro Val Lys Met Ala Thr Asn Ala Val Leu Cys Cys Pro Pro
45 50 55 60
atc gca tta aga aat ttg atc ata ata aca tgg gaa ata atc ctg aga 522
Ile Ala Leu Arg Asn Leu Ile Ile Ile Thr Trp Glu Ile Ile Leu Arg
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Gly Gln Pro Ser Cys Thr Lys Ala Tyr Lys Lys Glu Thr Asn Glu Thr

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95 100 105

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125 130 135 140

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Asn Arg Asn Arg Thr Ala Val Cys Lys Ala Val Ala Gly Lys Pro Ala
160 165 170

gcg cat atc tcc tgg atc cca gag ggc gat tgt gcc act aag caa gaa 858
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175 180 185

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Tyr Trp Ser Asn Gly Thr Val Thr Val Lys Ser Thr Cys His Trp Glu
190 195 200

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Val His Asn Val Ser Thr Val Thr Cys His Val Ser His Leu Thr Gly
205 210 215 220

aac aag agt ctg tac ata gag cta ctt cct gtt cca ggt gcc aaa aaa 1002
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225 230 235

atc agc aaa att ata tat tcc ata tat cat cct tac tat tat tat tta 1050
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240 245 250

gac cat cgt ggg att cat ttg gtt gtt gaa agt caa tgg ctg cag aaa 1098
Asp His Arg Gly Ile His Leu Val Val Glu Ser Gln Trp Leu Gln Lys
255 260 265

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Ile

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aggcatctga ggcattacaa agtgaagttg acacagacct ccatacttta taagttgttg 1271

gactctagta ccaagaaaca acaacaaacg agatacatta taattactgt ctgattttct 1331

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7

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10 15 20
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25 30 35
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55 60 65 70
Trp Glu Ile Ile Leu Arg Gly Gln Pro Ser Cys Thr Lys Ala Tyr Lys
75 80 85
Lys Glu Thr Asn Glu Thr Lys Glu Thr Asn Cys Thr Asp Glu Arg Ile
90 95 100
Thr Trp Val Ser Arg Pro Asp Gln Asn Ser Asp Leu Gln Ile Arg Thr
105 110 115
Val Ala Ile Thr His Asp Gly Tyr Tyr Arg Cys Ile Met Val Thr Pro
120 125 130
Asp Gly Asn Phe His Arg Gly Tyr His Leu Gln Val Leu Val Thr Pro
135 140 145 150
Glu Val Thr Leu Phe Gln Asn Arg Asn Arg Thr Ala Val Cys Lys Ala
155 160 165
Val Ala Gly Lys Pro Ala Ala His Ile Ser Trp Ile Pro Glu Gly Asp
170 175 180
Cys Ala Thr Lys Gln Glu Tyr Trp Ser Asn Gly Thr Val Thr Val Lys
185 190 195
Ser Thr Cys His Trp Glu Val His Asn Val Ser Thr Val Thr Cys His
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Val Ser His Leu Thr Gly Asn Lys Ser Leu Tyr Ile Glu Leu Leu Pro

215 220 225 230
 Val Pro Gly Ala Lys Lys Ile Ser Lys Ile Ile Tyr Ser Ile Tyr His
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 10 15 20

 gtg tct gta cag ata ggt aca aag gct ctg ctc tgc tgc ttt tct att 195
 Val Ser Val Gln Ile Gly Thr Lys Ala Leu Leu Cys Cys Phe Ser Ile
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 cca ctg aca aaa gca gta tta atc aca tgg ata ata aag ctc aga ggc 243
 Pro Leu Thr Lys Ala Val Leu Ile Thr Trp Ile Ile Lys Leu Arg Gly
 40 45 50

 ctg cca tcc tgc aca ata gca tac aaa gta gat aca aag acc aat gaa 291
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 55 60 65

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 70 75 80 85

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9

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Ile	Ser	Trp	Ser	Pro	Asp	Gly	Asp	Cys	Val	Thr	Thr	Ser	Glu	Ser	His	
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Asn	Val	Ser	Asp	Val	Ser	Cys	Ile	Val	Ser	His	Leu	Thr	Gly	Asn	Gln	
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Ser	Leu	Ser	Ile	Glu	Leu	Ser	Arg	Gly	Gly	Asn	Gln	Ser	Leu	Arg	Pro	
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Tyr	Ile	Pro	Tyr	Ile	Ile	Pro	Ser	Ile	Ile	Ile	Leu	Ile	Ile	Ile	Gly	
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Cys	Ile	Cys	Leu	Leu	Lys	Ile	Ser	Gly	Phe	Arg	Lys	Cys	Lys	Leu	Pro	
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Lys	Leu	Glu	Ala	Thr	Ser	Ala	Ile	Glu	Glu	Asp	Glu	Met	Gln	Pro	Tyr	
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Ala	Ser	Tyr	Thr	Glu	Lys	Ser	Asn	Pro	Leu	Tyr	Asp	Thr	Val	Thr	Lys	
				265				270					275			
gtg	gag	gca	ttt	cca	gta	tca	caa	ggc	gaa	gtc	aat	ggc	aca	gac	tgc	963
Val	Glu	Ala	Phe	Pro	Val	Ser	Gln	Gly	Glu	Val	Asn	Gly	Thr	Asp	Cys	
				280				285				290				
ctt	act	ttg	tcg	gcc	att	gga	atc	tagaac	caag	aaaaaagaag	tcaagagaca					1017
Leu	Thr	Leu	Ser	Ala	Ile	Gly	Ile									
				295		</										

10
 ttggaagttt ggaatctctg ctgctacctg ttaatttttag gaagaactga tttaattatt 1197
 acaaagaaag cacatgggta tgggtgaaata tcaagttgtg caataaagta tgatgaaaac 1257
 tgagtttctt caagaaataa ctgcaggagg aacaatcatc actaaagaat ttcattgtgag 1317
 ttcttacaaa aaaattccta tgtatacatg actatgggtat gtgtgtccaa ttacatgttt 1377
 atttacaaat gtgtatatat gcacacattt gcttttcagg acatctcctt gtaaaaaaca 1437
 cactggagtt ttggatttat aaaagcttat aaagttagca ttggagatat ttt 1490

<210> 6
 <211> 326
 <212> PRT
 <213> Unknown

<400> 6
 Met Phe Cys Phe Trp Arg Thr Ser Ala Leu Ala Val Leu Leu Ile Trp
 -25 -20 -15 -10
 Gly Val Phe Val Ala Gly Ser Ser Cys Thr Asp Lys Asn Gln Thr Thr
 -5 -1 1 5
 Gln Asn Asn Ser Ser Ser Pro Leu Thr Gln Val Asn Thr Thr Val Ser
 10 15 20
 Val Gln Ile Gly Thr Lys Ala Leu Leu Cys Cys Phe Ser Ile Pro Leu
 25 30 35
 Thr Lys Ala Val Leu Ile Thr Trp Ile Ile Lys Leu Arg Gly Leu Pro
 40 45 50 55
 Ser Cys Thr Ile Ala Tyr Lys Val Asp Thr Lys Thr Asn Glu Thr Ser
 60 65 70
 Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro Asp His Ser Pro
 75 80 85
 Glu Leu Gln Ile Ser Ala Val Thr Leu Gln His Glu Gly Thr Tyr Thr
 90 95 100
 Cys Glu Thr Val Thr Pro Glu Gly Asn Phe Glu Lys Asn Tyr Asp Leu
 105 110 115
 Gln Val Leu Val Pro Pro Glu Val Thr Tyr Phe Pro Glu Lys Asn Arg
 120 125 130 135
 Ser Ala Val Cys Glu Ala Met Ala Gly Lys Pro Ala Ala Gln Ile Ser
 140 145 150
 Trp Ser Pro Asp Gly Asp Cys Val Thr Thr Ser Glu Ser His Ser Asn
 155 160 165
 Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu Gln Asn Asn Val
 170 175 180
 Ser Asp Val Ser Cys Ile Val Ser His Leu Thr Gly Asn Gln Ser Leu

[illegible]

<210>	7
<211>	1010
<212>	DNA
<213>	Unknown

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<220>
<223> Description of Unknown Organism:primate; surmised
      homo sapiens
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<220>  
<221> CDS  
<222> (1) .. (750)
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<400> 7																
atg	ggt	gga	aag	cag	atg	aca	cag	aac	tat	tca	aca	att	ttt	gca	gaa	48
Met	Gly	Gly	Lys	Gln	Met	Thr	Gln	Asn	Tyr	Ser	Thr	Ile	Phe	Ala	Glu	
1				5				10						15		
ggt	aac	att	tca	cag	cct	gta	ctg	atg	gat	ata	aat	gct	gtg	ctt	tgt	96
Gly	Asn	Ile	Ser	Gln	Pro	Val	Leu	Met	Asp	Ile	Asn	Ala	Val	Leu	Cys	
		20						25				30				
tgc	cct	cct	att	gca	tta	aga	aat	ttg	atc	ata	ata	aca	tgg	gaa	ata	144
Cys	Pro	Pro	Ile	Ala	Leu	Arg	Asn	Leu	Ile	Ile	Ile	Thr	Trp	Glu	Ile	
		35				40						45				
atc	ctg	aga	ggc	cag	cct	tcc	tgc	aca	aaa	gcc	tac	aag	aaa	gaa	aca	192
Ile	Leu	Arg	Gly	Gln	Pro	Ser	Cys	Thr	Lys	Ala	Tyr	Lys	Lys	Glu	Thr	
50						55				60						
aat	gag	acc	aag	gaa	acc	aac	tgt	act	gtt	gag	aga	ata	acc	tgg	gtc	240
Asn	Glu	Thr	Lys	Glu	Thr	Asn	Cys	Thr	Val	Glu	Arg	Ile	Thr	Trp	Val	
65				70						75				80		
tct	aga	cct	gat	cag	aat	tcg	gac	ctt	cag	att	cgt	ccg	gtg	gac	acc	288

[illegible]

<210>	9
<211>	1085
<212>	DNA
<213>	Unknown

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<220>
<223> Description of Unknown Organism:rodent; surmised
      mus musculus
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<220>
<221> CDS

14

<222> (1)..(582)

<400> 9

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aga ggc cag cct tcc tgc ata atg gcc tac aaa gta gaa aca aag gag 48
Arg Gly Gln Pro Ser Cys Ile Met Ala Tyr Lys Val Glu Thr Lys Glu
1 5 10 15

acc aat gaa acc tgc ttg ggc agg aac atc acc tgg gcc tcc aca cct 96
Thr Asn Glu Thr Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro
20 25 30

gac cac att cct gac ctt cag atc agt gcg gtg gcc ctc cag cat gag 144
Asp His Ile Pro Asp Leu Gln Ile Ser Ala Val Ala Leu Gln His Glu
35 40 45

ggg aat tac tta tgt gag ata aca aca cct gaa ggg aat ttc cat aaa 192
Gly Asn Tyr Leu Cys Glu Ile Thr Thr Pro Glu Gly Asn Phe His Lys
50 55 60

gtc tat gac ctc caa gtg ctg gtg ccc cct gaa gta acc tac ttt ctc 240
Val Tyr Asp Leu Gln Val Leu Val Pro Pro Glu Val Thr Tyr Phe Leu
65 70 75 80

ggg gaa aat aga act gca gtt tgt gag gca atg gca ggc aag cct gct 288
Gly Glu Asn Arg Thr Ala Val Cys Glu Ala Met Ala Gly Lys Pro Ala
85 90 95

gca cag atc tct tgg act cca gat ggg gac tgt gtc act aag agt gag 336
Ala Gln Ile Ser Trp Thr Pro Asp Gly Asp Cys Val Thr Lys Ser Glu
100 105 110

tca cac agc aat ggc act gtg act gtc agg agc act tgc cac tgg gag 384
Ser His Ser Asn Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu
115 120 125

cag aac aat gtg tct gct gtg tcc tgc att gtc tct cat tcg act ggt 432
Gln Asn Asn Val Ser Ala Val Ser Cys Ile Val Ser His Ser Thr Gly
130 135 140

aat cag tct ctg tcc ata gaa ctg agt aga ggt acc acc agc acc acc 480
Asn Gln Ser Leu Ser Ile Glu Leu Ser Arg Gly Thr Thr Ser Thr Thr
145 150 155 160

cct tcc ttg ctg acc att ctc tac gtg aaa atg gtc ctt ttg ggg att 528
Pro Ser Leu Leu Thr Ile Leu Tyr Val Lys Met Val Leu Leu Gly Ile
165 170 175

att ctt ctt aaa gtg gga ttt gct ttc ttc cag aag aga aat gtt acc 576
Ile Leu Leu Lys Val Gly Phe Ala Phe Phe Gln Lys Arg Asn Val Thr
180 185 190

aga aca tgaatatcca gatttctgga agctcattag tctgatgaca cataccagaa 632
Arg Thr

aacagcattt gtaatcaact ttctcattgg aatccagctt acccgteccct gctgtcttca 692

tgtttgtag acactcacct ccaaattctt aactgagaag ggctcctgtc taaaggaaat 752

atgggggacaa attgtggagc atagacaaaa agaaaggcca tccagagact gccccaccta 812

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<210> 11

<400> 11																
ggcagcagatt acgattttgtg cttaacctga ctccactcca g atg cat gct ttg ggg																56
Met His Ala Leu Gly																
-25																
agg act ctg gct ttg atg tta ctc atc ttc atc act att ttg gtg cct																104
Arg Thr Leu Ala Leu Met Leu Leu Ile Phe Ile Thr Ile Leu Val Pro																
-20 -15 -10 -5																
gag tca agt tgt tca gtg aaa gga cgg gag gag atc cca ccg gat gat																152
Glu Ser Ser Cys Ser Val Lys Gly Arg Glu Glu Ile Pro Pro Asp Asp																
-1 1 5 10																
tca ttt cct ttt tca gat gat aat atc ttc cct gat gga gtg ggc gtc																200
Ser Phe Pro Phe Ser Asp Asp Asn Ile Phe Pro Asp Gly Val Gly Val																
15 20 25																
acc atg gag att gag att atc act cca gtg tct gta cag ata ggt atc																248
Thr Met Glu Ile Glu Ile Ile Thr Pro Val Ser Val Gln Ile Gly Ile																
30 35 40																
aag gct cag ctt ttc tgt cat cct agt cca tca aaa gaa gca aca ctt																296
Lys Ala Gln Leu Phe Cys His Pro Ser Pro Ser Lys Glu Ala Thr Leu																
45 50 55 60																
aga ata tgg gaa ata act ccc aga gac tgg cct tcc tgc aga cta ccc																344
Arg Ile Trp Glu Ile Thr Pro Arg Asp Trp Pro Ser Cys Arg Leu Pro																
65 70 75																
tac aga gca gag ttg cag cag atc agt aaa aaa atc tgt act gag aga																392
Tyr Arg Ala Glu Leu Gln Gln Ile Ser Lys Lys Ile Cys Thr Glu Arg																
80 85 90																
gga acc act agg gtc cct gca cat cac cag agt tct gac ctt ccc atc																440
Gly Thr Thr Arg Val Pro Ala His His Gln Ser Ser Asp Leu Pro Ile																
95 100 105																
aaa tca atg gcc ctc aag cat gat ggg cat tac tca tgt cgg ata gaa																488
Lys Ser Met Ala Leu Lys His Asp Gly His Tyr Ser Cys Arg Ile Glu																
110 115 120																
aca aca gat ggg att ttc caa gag aga cat agc atc caa gtg cca ggg																536
Thr Thr Asp Gly Ile Phe Gln Glu Arg His Ser Ile Gln Val Pro Gly																

$$\begin{aligned} \frac{\partial^2 \mathcal{L}}{\partial \theta^2} &= \frac{\partial}{\partial \theta} \left(\frac{\partial \mathcal{L}}{\partial \theta} \right) = \frac{\partial}{\partial \theta} \left(\frac{\partial}{\partial \theta} \left(\frac{1}{2} \theta^2 \right) \right) = \frac{\partial}{\partial \theta} (\theta) = 1 \\ \frac{\partial^2 \mathcal{L}}{\partial \theta \partial \theta} &= \frac{\partial}{\partial \theta} \left(\frac{\partial \mathcal{L}}{\partial \theta} \right) = \frac{\partial}{\partial \theta} (\theta) = 1 \end{aligned}$$

Thr Ile Leu Val Pro Glu Ser Ser Cys Ser Val Lys Gly Arg Glu Glu
 -5 -1 1 5
 Ile Pro Pro Asp Asp Ser Phe Pro Phe Ser Asp Asp Asn Ile Phe Pro
 10 15 20
 Asp Gly Val Gly Val Thr Met Glu Ile Glu Ile Ile Thr Pro Val Ser
 25 30 35
 Val Gln Ile Gly Ile Lys Ala Gln Leu Phe Cys His Pro Ser Pro Ser
 40 45 50 55
 Lys Glu Ala Thr Leu Arg Ile Trp Glu Ile Thr Pro Arg Asp Trp Pro
 60 65 70
 Ser Cys Arg Leu Pro Tyr Arg Ala Glu Leu Gln Gln Ile Ser Lys Lys
 75 80 85
 Ile Cys Thr Glu Arg Gly Thr Thr Arg Val Pro Ala His His Gln Ser
 90 95 100
 Ser Asp Leu Pro Ile Lys Ser Met Ala Leu Lys His Asp Gly His Tyr
 105 110 115
 Ser Cys Arg Ile Glu Thr Thr Asp Gly Ile Phe Gln Glu Arg His Ser
 120 125 130 135
 Ile Gln Val Pro Gly Glu Asn Arg Thr Val Val Cys Glu Ala Ile Ala
 140 145 150
 Ser Lys Pro Ala Met Gln Ile Leu Trp Thr Pro Asp Glu Asp Cys Val
 155 160 165
 Thr Lys Ser Lys Ser His Asn Asp Thr Met Ile Val Arg Ser Lys Cys
 170 175 180
 His Arg Glu Lys Asn Asn Gly His Ser Val Phe Cys Phe Ile Ser His
 185 190 195
 Leu Thr Asp Asn Trp Ile Leu Ser Met Glu Gln Asn Arg Gly Thr Thr
 200 205 210 215
 Ser Ile Leu Pro Ser Leu Leu Ser Ile Leu Tyr Val Lys Leu Ala Val
 220 225 230
 Thr Val Leu Ile Val Gly Phe Ala Phe Phe Gln Lys Arg Asn Tyr Phe
 235 240 245
 Arg Val Pro Glu Gly Ser
 250

<210> 13
 <211> 981
 <212> DNA
 <213> reverse translation

<220>
 <221> misc_feature

<222> (1)..(981)

<223> n may be a, c, g, or t

<400> 13

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gngarwsnw sntgyccnga yaaraaycar acnatgcara ayaaywsnws nacnatgacn 120
gargtnaaya cnaengtntt ygtncaratg ggnaaraarg cnytnytntg ytgyccnwsn 180
athwsnytna cnaargtnat hytnathacn tggacnatha cnytnmgngg ncarccnwsn 240
tgyathathw sntayaargc ngayacnmgn garacncayg arwsnaaytg ywsngaymgn 300
wsnathacnt gggcnwsnac nccngayytn gncngayy tncarathws ngcngtngcn 360
ytncarcayg arggnmgnta ywsntgygay athgcngtnc cngayggnaa yttycaraay 420
athtaygayy tncargtnyt ngtnccncn gargtnacnc ayttyccngg ngaraaymgn 480
acngcngtnt gygargcnat hgcnggnaar ccngcngcnc arathwsntg gacncngay 540
ggngaytgyg tngcnaaraa ygarwsncay wsnaayggna cngtnacngt nmgnwsnacn 600
tgycaytggg arcarwsnca ygtnwsngtn gtnttytgyg tngtnwsnca yytnacnacn 660
ggnaaycarw snytnwsnat hgarytnggn mgngggngng aycarytnyt nggnwsntay 720
athcartaya thathccnws nathathath ytnathatha thggntgyat htgyytnytn 780
aarathwsng gntgymgnaa rtgyaarytn ccnaarwsng gngcnacncc ngayathgar 840
gargaygara tgcarccnta ygcnsntay acngaraarw snaayccnyt ntaygayacn 900
gtnacnacna cngargcnca yccngcnwsn carggnaarg tnaayggnac ngaytgyytn 960
acnytnwsng cnatgggnat h 981

<210> 14

<211> 885

<212> DNA

<213> reverse translation

<220>

<221> misc_feature

<222> (1)..(885)

<223> n may be a, c, g, or t

<400> 14

atgytntgyt cntggmgnc ngcnaayytn ggnytnytny tnathytnac nathttyytn 60
gtngcngarg cngarggngc ngcncarccn aayaaywsny tnatgytnca racnwsnaar 120
garaaycayg cnytngcnws nwsnwsnytn tgyatggayg araarcarat hacncaraay 180
taywsnaarg tnytngcnga rgtnaayacn wsntggccng tnaaratggc nacnaaygcn 240
gtnytnntgyt gyccnccnat hgcnytnmgn aayytnatha thathacntg ggarathath 300

ytnmgnggnc arccnwsntg yacnaargcn tayaaraarg aracnaayga racnaargar 360
 acnaaytgya cngaygarmg nathacntgg gtnwsnmgnc cngaycaraa ywsngayytn 420
 carathmgna cngtngcnat hacncaygay ggntaytaym gntgyathat ggtnacnccn 480
 gayggnaayt tycaymgngg ntaycayytn cargtnytng tnacncnga rgtnacnytn 540
 ttycaraaym gnaaymgna ngcngtntgy aargcngtng cnggnaarcc ngcngcncay 600
 athwsntgga thccngargg ngaytgygcn acnaarcarg artaytggws naayggnaacn 660
 gtnacngtna arwsnacntg ycaytgggar gtncayaayg tnwsnacngt nacntgygay 720
 gtnwsncayy tnacnggnaa yaarwsnytn tayathgary tnytnccngt nccngngncn 780
 aaraarathw snaarathat htaywsnath taycaycct aytaytayta yytngaygay 840
 mgnggnathc ayytngtngt ngarwsncar tggytncara arath 885

<210> 15
 <211> 978
 <212> DNA
 <213> reverse translation

 <220>
 <221> misc_feature
 <222> (1)..(978)
 <223> n may be a, c, g, or t

<400> 15
 atgttytgyt tytggmgna nwsngcnnytn gngtynytn tnathtgggg ngtnnttygtn 60
 gnggnwnsw sntgyacnga yaaraaycar acnacncara ayaaywsnws nwsnccnytn 120
 acncargtna ayacnacngt nwsngtncar athggnaacna argcnnytn ntgytgytty 180
 wsnathccny tnacnaargc ngtnytnath acntggatha thaarytnmg nggnytnccn 240
 wsntgyacna thgcntayaa rgtngayacn aaracnaayg aracnwsntg yytnggngmn 300
 aayathacnt gggcnwsnac nccngaygay wsncngary tncarathws ngcngtnacn 360
 ytnrcarcayg arggnacnta yacntgygar acngtnacnc cngarggnaa yttygaraar 420
 aaytaygayy tncargtnyt ngtnccnccn gargtnacnt ayttyccnga raaraaymgn 480
 wsnngcngtnt gygargcnat ggcnggnaar ccngcngcnc arathwsntg gwsnccngay 540
 ggngaytgyg tnacnacnws ngarwsncay wsnaayggna cngtnacngt nmgnwsnacn 600
 tgycaytggg arcaraayaa ygtnwsngay gtnwsntgya thgtnwsnca yytnacnggn 660
 aaycarwsny tnwsnathga rytwnsmgn ggnggnaayc arwsnytnmg nccntayath 720
 ccntayatha thccnwsnat hathathytn athathathg gntgyathtg yytnytnaar 780

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<400> 17
mngngncarc cnwsntgyat hatggcntay aargtngara cnaargarac naaygaracn 60
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tgyytngggnm gnaayathac ntgggcnwsn acnccngayc ayathccnga yytnccarath 120
wsngcngtng cnytnccarca ygarggnaay tayytntgyg arathacnac nccngarggn 180
aayttycaya argtntayga yytnccargtn ytngtnccnc cngargtnac ntaytttytn 240
ggngaraaym gnacngcngt ntgygargcn atggcnggna arccngcngc nccarathwsn 300
tggacnccng ayggngaytg ygtnacnaar wsgarwsnc aywsnaaygg nacngtnacn 360
gtngmgnwsna cntgycaytg ggarcaraay aaygtngwsng cngtnwsntg yathgtngwsn 420
caywsnacng gnaaycarws nytnwsnath garytnwsnm gnggnacnac nwsnacnacn 480
ccnwsnytny tnacnathyt ntaygtnaar atggtnytny tnggnathat hytnytnaar 540
gtnggnttyg cnttyttyca raarmgnaay gtnacnmgna cn 582

<210> 18
<211> 834
<212> DNA
<213> reverse translation
<220>
<221> misc_feature
<222> (1)..(834)
<223> n may be a, c, g, or t

<400> 18
atgcaygcny tnggngmgnac nytnngcnytn atgytnytna thtttyathac nathytngtn 60
ccngarwsnw sntgywsngt naarggngmgn gargarathc cncnccngayga ywsnttyccn 120
ttywsngayg ayaayathtt yccngayggn gtnggngtna cnatggarat hgarathath 180
acnccngtnw sngtnccarat hgggnathaar gcncarytn tytgycaycc nwsnccnwsn 240
aargargcna cnytnmgnat htgggarath acnccnmgng aytggccnws ntgygmnytn 300
ccntaymgng cngarytnca rccarathwsn aaraaratht gyacngarmg nggnacnacn 360
mgngtnccng cncaycayca rwsnwsngay ytnccnatha arwsnatggc nytnaarcay 420
gayggncayt aywsntgyng nathgaracn acngayggna thttycarga rmngncaywsn 480
athcargtn cnggngaraa ymgngacngtn gtntgygarg cnathgcnws naarcngcn 540
atgcarathy tntggacncc ngaygargay tgygtnacna arwsnaarws ncayaygay 600
acnatgathg tnmgnwsnaa rtgycaymgng garaaraaya ayggncayws ngtnnttytg 660
ttyathwsnc ayytnacnga yaaytgath ytnwsnatgg arcaraaymg nggnacnacn 720
wsnathytnc cnwsnytnyt nwsnathytnt taygtnaary tngcngtnac ngtnytnath 780
gtnggnttyg cnttyttyca raarmgnaay tayttymgng tncnccngargg nwsn 834

<210> 19
 <211> 1047
 <212> DNA
 <213> Unknown

<220>
 <223> Description of Unknown Organism:primate; surmised
 homo sapiens

<220>
 <221> CDS
 <222> (1)..(1044)
 <220>
 <221> mat_peptide
 <222> (79)..(1044)

<400> 19
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 Met Leu Cys Pro Trp Arg Thr Ala Asn Leu Gly Leu Leu Leu Ile Leu
 -25 -20 -15
 act atc ttc tta gtg gcc gaa gcg gag ggt gct gct caa cca aac aac 96
 Thr Ile Phe Leu Val Ala Glu Ala Glu Gly Ala Ala Gln Pro Asn Asn
 -10 -5 -1 1 5
 tca tta atg ctg caa act agc aag gag aat cat gct tta gct tca agc 144
 Ser Leu Met Leu Gln Thr Ser Lys Glu Asn His Ala Leu Ala Ser Ser
 10 15 20
 agt tta tgt atg gat gaa aaa cag att aca cag aac tac tcg aaa gta 192
 Ser Leu Cys Met Asp Glu Lys Gln Ile Thr Gln Asn Tyr Ser Lys Val
 25 30 35
 ctc gca gaa gtt aac act tca tgg cct gta aag atg gct aca aat gct 240
 Leu Ala Glu Val Asn Thr Ser Trp Pro Val Lys Met Ala Thr Asn Ala
 40 45 50
 gtg ctt tgt tgc cct cct atc gca tta aga aat ttg atc ata ata aca 288
 Val Leu Cys Cys Pro Pro Ile Ala Leu Arg Asn Leu Ile Ile Ile Thr
 55 60 65 70
 tgg gaa ata atc ctg aga ggc cag cct tcc tgc aca aaa gcc tac agg 336
 Trp Glu Ile Ile Leu Arg Gly Gln Pro Ser Cys Thr Lys Ala Tyr Arg
 75 80 85
 aaa gaa aca aat gag acc aag gaa acc aac tgt act gat gag aga ata 384
 Lys Glu Thr Asn Glu Thr Lys Glu Thr Asn Cys Thr Asp Glu Arg Ile
 90 95 100
 acc tgg gtc tcc aga cct gat cag aat tcg gac ctt cag att cgt cca 432
 Thr Trp Val Ser Arg Pro Asp Gln Asn Ser Asp Leu Gln Ile Arg Pro
 105 110 115
 gtg gcc atc act cat gac ggg tat tac aga tgc ata atg gta aca cct 480
 Val Ala Ile Thr His Asp Gly Tyr Tyr Arg Cys Ile Met Val Thr Pro
 120 125 130

24
gat ggg aat ttc cat cgt gga tat cac ctc caa gtg tta gtt aca cct 528
Asp Gly Asn Phe His Arg Gly Tyr His Leu Gln Val Leu Val Thr Pro
135 140 145 150
gaa gtg acc ctg ttt caa aac agg aat aga act gca gta tgc aag gca 576
Glu Val Thr Leu Phe Gln Asn Arg Asn Arg Thr Ala Val Cys Lys Ala
155 160 165
gtt gca ggg aag cca gct gcg cag atc tcc tgg atc cca gag ggc gat 624
Val Ala Gly Lys Pro Ala Ala Gln Ile Ser Trp Ile Pro Glu Gly Asp
170 175 180
tgt gcc act aag caa gaa tac tgg agc aat ggc aca gtg act gtt aag 672
Cys Ala Thr Lys Gln Glu Tyr Trp Ser Asn Gly Thr Val Thr Val Lys
185 190 195
agt aca tgc cac tgg gag gtc cac aat gtg tct acc gtg acc tgc cac 720
Ser Thr Cys His Trp Glu Val His Asn Val Ser Thr Val Thr Cys His
200 205 210
gtc tcc cat ttg act ggc aac aag agt ctg tac ata gag cta ctt cct 768
Val Ser His Leu Thr Gly Asn Lys Ser Leu Tyr Ile Glu Leu Leu Pro
215 220 225 230
gtt cca ggt gcc aaa aaa tca gca aaa tta tat att cca tat atc atc 816
Val Pro Gly Ala Lys Lys Ser Ala Lys Leu Tyr Ile Pro Tyr Ile Ile
235 240 245
ctt act att att att ttg acc atc gtg gga ttc att tgg ttg ttg aaa 864
Leu Thr Ile Ile Ile Leu Thr Ile Val Gly Phe Ile Trp Leu Leu Lys
250 255 260
gtc aat ggc tgc aga aaa tat aaa ttg aat aaa aca gaa tct act cca 912
Val Asn Gly Cys Arg Lys Tyr Lys Leu Asn Lys Thr Glu Ser Thr Pro
265 270 275
gtt gtt gag gag gat gaa atg cag ccc tat gcc agc tac aca gag aag 960
Val Val Glu Glu Asp Glu Met Gln Pro Tyr Ala Ser Tyr Thr Glu Lys
280 285 290
aac aat cct ctc tat gat act aca aac aag gtg aag gca tct cag gca 1008
Asn Asn Pro Leu Tyr Asp Thr Thr Asn Lys Val Lys Ala Ser Gln Ala
295 300 305 310
tta caa agt gaa gtt gac aca gac ctc cat act tta taa 1047
Leu Gln Ser Glu Val Asp Thr Asp Leu His Thr Leu
315 320

<210> 20
<211> 348
<212> PRT
<213> Unknown

<400> 20
Met Leu Cys Pro Trp Arg Thr Ala Asn Leu Gly Leu Leu Leu Ile Leu
-25 -20 -15

Thr Ile Phe Leu Val Ala Glu Ala Glu Gly Ala Ala Gln Pro Asn Asn

26
Leu Gln Ser Glu Val Asp Thr Asp Leu His Thr Leu
315 320

<210> 21
<211> 1044
<212> DNA
<213> reverse translation

<220>
<221> misc_feature
<222> (1)..(1044)
<223> n may be a, c, g, or t

<400> 21
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gtngcngarg cngarggngc ngcncarccn aayaaywsny tnatgytnca racnwsnaar 120
garaaycayg cnytnngcws nwsnwsnytn tgyatggayg araarcarat hacncaraay 180
taywsnaarg tnytnngcga rgtnaayaen wsntggccng tnaaratggc nacnaaygcn 240
gtnytnytny gycncncnat hgcnytnmgn aayytnatha thathacntg ggarathath 300
ytnmngggnc arccnwsntg yacnaargcn taymngnaarg aracnaayga racnaargar 360
acnaaytgya cngaygarmg nathacntgg gtnwsnmgnc cngaycaraa ywsngayytn 420
carathmgnc cngtnngcnat hacncaygay ggntaytaym gntgyathat ggtnacnccn 480
gayggnaayt tycaymgngg ntaycayytn cargtnytny tnacncnga rgtnacnytn 540
ttypcaraaym gnaaymgnc ngcngtnytn aargcngtny cnggnaarcc ngcngncar 600
athwsntgga thccngargg ngaytgygcn acnaarcarg artaytggws naayggnaen 660
gtnacngtna arwsnacntg ycaytgggar gtncayaayg tnwsnacngt nacntgygay 720
gtwnsncaay tnacnggnaa yaarwsnytn tayathgary tnytnccngt nccnggngcn 780
aaraarwsng cnaarytna yathccntay athathytna cnathathat hytnacnath 840
gtnggnttya thtgyytny naargtnaay ggntgymgna artayaaryt naayaaracn 900
garwsnacnc cngtnngtna rgargaygar atgcarccnt aygcnwsnta yacngaraar 960
aayaayccny tntaygayac nacnaayaar gtnaargcnw sncargcnytn ncarwsngar 1020
gtngayacng ayytncaayac nytn 1044

<210> 22
<211> 813
<212> DNA
<213> Unknown

<220>
<223> Description of Unknown Organism:rodent; surmised

mus musculus

 $\langle 220 \rangle$

<221> CDS

 $\langle 222 \rangle \quad (1) \dots (810)$ $\langle 220 \rangle$

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<221> mat_peptide
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<222> (76) .. (810)

<400> 22

atg cat gct ctg ggg agg att ccg act ttg act ttg ctg atc ttc atc 48
Met His Ala Leu Gly Arg Ile Pro Thr Leu Thr Leu Leu Ile Phe Ile
-25 -20 -15 -10

aat att ttt gtg tct ggg tca agt tgt act gat gag aat caa aca ata 96
Asn Ile Phe Val Ser Gly Ser Ser Cys Thr Asp Glu Asn Gln Thr Ile
-5 -1 1 5

cag aat gac agt tca tct tct ctg aca caa gtt aac act aca atg tct 144
Gln Asn Asp Ser Ser Ser Ser Leu Thr Gln Val Asn Thr Thr Met Ser
10 15 20

gta cag atg gat aaa aag gct ctg ctc tgc tgc ttt tct agt cca ctg 192
Val Gln Met Asp Lys Lys Ala Leu Leu Cys Cys Phe Ser Ser Pro Leu
25 30 35

ata aat gca gta tta atc aca tgg ata ata aaa cac aga cac ctg cct 240
Ile Asn Ala Val Leu Ile Thr Trp Ile Ile Lys His Arg His Leu Pro
40 45 50 55

tcc tgc aca ata gca tac aac cta gat aaa aag acc aat gaa acc agc 288
Ser Cys Thr Ile Ala Tyr Asn Leu Asp Lys Lys Thr Asn Glu Thr Ser
60 65 70

tgc ttg ggc agg aac atc acc tgg gcc tcc aca cct gac cac agt cct 336
Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro Asp His Ser Pro
75 80 85

gaa ctt cag atc agt gca gtg gcc ctc cag cat gag ggg act tac aca 384
Glu Leu Gln Ile Ser Ala Val Ala Leu Gln His Glu Gly Thr Tyr Thr
90 95 100

tgt gag ata gta aca cct gaa ggg aat tta gaa aaa gtc tat gac ctc 432
Cys Glu Ile Val Thr Pro Glu Gly Asn Leu Glu Lys Val Tyr Asp Leu
105 110 115

caa gtg ctg gtg ccc cct gag gta acc tac ttt cca ggg aaa aac aga 480
Gln Val Leu Val Pro Pro Glu Val Thr Tyr Phe Pro Gly Lys Asn Arg
120 125 130 135

act gca gtc tgt gag gca atg gca ggc aag cct gct gca cag atc tct 528
Thr Ala Val Cys Glu Ala Met Ala Gly Lys Pro Ala Ala Gln Ile Ser
140 145 150

tgg act cca gat ggg gac tgt gtc act aag agt gag tca cac agc aat 576
 Trp Thr Pro Asp Gly Asp Cys Val Thr Lys Ser Glu Ser His Ser Asn
 155 160 165

28																
ggc	act	gtg	act	gtc	agg	agc	acg	tgc	cac	tgg	gag	cag	aac	aat	gtg	624
Gly	Thr	Val	Thr	Val	Arg	Ser	Thr	Cys	His	Trp	Glu	Gln	Asn	Asn	Val	
		170				175						180				
tct	gtt	gtg	tcc	tgc	tta	gtc	tct	cat	tcg	act	ggt	aat	cag	tct	ctg	672
Ser	Val	Val	Ser	Cys	Leu	Val	Ser	His	Ser	Thr	Gly	Asn	Gln	Ser	Leu	
		185				190						195				
tcc	ata	gaa	ctg	agt	caa	ggc	aca	atg	acc	acc	ccc	cgt	tcc	ttg	ctg	720
Ser	Ile	Glu	Leu	Ser	Gln	Gly	Thr	Met	Thr	Thr	Pro	Arg	Ser	Leu	Leu	
200					205						210				215	
acc	att	ctc	tat	gtg	aaa	atg	gcc	ctt	ttg	gtg	att	att	ctt	ctt	aac	768
Thr	Ile	Leu	Tyr	Val	Lys	Met	Ala	Leu	Leu	Val	Ile	Ile	Leu	Leu	Asn	
				220						225				230		
gta	gga	ttt	gct	ttc	ttc	cag	aag	aga	aat	ttt	gcc	aga	aca	tga		813
Val	Gly	Phe	Ala	Phe	Phe	Gln	Lys	Arg	Asn	Phe	Ala	Arg	Thr			
		235						240				245				

<210>	23
<211>	270
<212>	PRT
<213>	Unknown

<400> 23

Met His Ala Leu Gly Arg Ile Pro Thr Leu Thr Leu Leu Ile Phe Ile
-25 -20 -15 -10

Asn Ile Phe Val Ser Gly Ser Ser Cys Thr Asp Glu Asn Gln Thr Ile
-5 -1 1 5

Gln Asn Asp Ser Ser Ser Ser Leu Thr Gln Val Asn Thr Thr Met Ser
10 15 20

Val Gln Met Asp Lys Lys Ala Leu Leu Cys Cys Phe Ser Ser Pro Leu
25 30 35

Ile Asn Ala Val Leu Ile Thr Trp Ile Ile Lys His Arg His Leu Pro
40 45 50 55

Ser Cys Thr Ile Ala Tyr Asn Leu Asp Lys Lys Thr Asn Glu Thr Ser
60 65 70

Cys Leu Gly Arg Asn Ile Thr Trp Ala Ser Thr Pro Asp His Ser Pro
75 80 85

Glu Leu Gln Ile Ser Ala Val Ala Leu Gln His Glu Gly Thr Tyr Thr
90 95 100

Cys Glu Ile Val Thr Pro Glu Gly Asn Leu Glu Lys Val Tyr Asp Leu
105 110 115

Gln Val Leu Val Pro Pro Glu Val Thr Tyr Phe Pro Gly Lys Asn Arg
120 125 130 135

Thr Ala Val Cys Glu Ala Met Ala Gly Lys Pro Ala Ala Gln Ile Ser
140 145 150

Trp Thr Pro Asp Gly Asp Cys Val Thr Lys Ser Glu Ser His Ser Asn
 155 160 165
 Gly Thr Val Thr Val Arg Ser Thr Cys His Trp Glu Gln Asn Asn Val
 170 175 180
 Ser Val Val Ser Cys Leu Val Ser His Ser Thr Gly Asn Gln Ser Leu
 185 190 195
 Ser Ile Glu Leu Ser Gln Gly Thr Met Thr Thr Pro Arg Ser Leu Leu
 200 205 210 215
 Thr Ile Leu Tyr Val Lys Met Ala Leu Leu Val Ile Ile Leu Leu Asn
 220 225 230
 Val Gly Phe Ala Phe Phe Gln Lys Arg Asn Phe Ala Arg Thr
 235 240 245

<210> 24
 <211> 810
 <212> DNA
 <213> reverse translation

<220>
 <221> misc_feature
 <222> (1)..(810)
 <223> n may be a, c, g, or t

<400> 24
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 acncargtna ayacnacnat gwsngtncar atggayaara argcnytnyt ntgytgytty 180
 wsnwsnccny tnathaaygc ngtnytnath acntggatha thaarcaymg ncayytncn 240
 wsntgyacna thgcntayaa yytngayaar aaracnaayg aracnwsntg yytnggnmgn 300
 aayathacnt gggcnwsnac nccngaycay wsncngary tncarathws ngcngtngcn 360
 ytnrcarayg arggnacnta yacntgygar athgtnacnc cngarggnaa yytngaraar 420
 gtntaygayy tncargtnyt ngtnccncn gargtnacnt ayttccngg naaraaymgn 480
 acngcngtnt gygargcnat ggcnggnaar ccngcngcnc arathwsntg gacncngay 540
 ggngaytgyg tnacnaarws ngarwsncay wsnaayggna cngtnacngt nmgnwsnacn 600
 tgycaytggg arcaraayaa ygtnwsngtn gtnwsntgyy tngtnwsnca ywsnacnggn 660
 aaycarwsny tnwsnathga rytnwsncar ggnacnatga cnacncnmg nwsnytnytn 720
 acnathytnt aygtnaarat ggcnytnytn gtnathathy tnytnaaygt nggnttygcn 780
 ttyttycara armgnaaytt ygcnmgnacn 810